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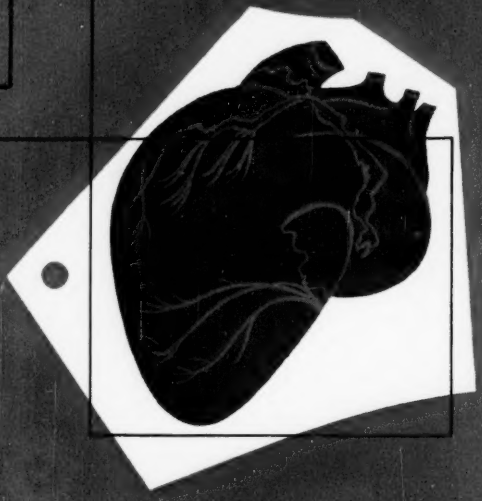

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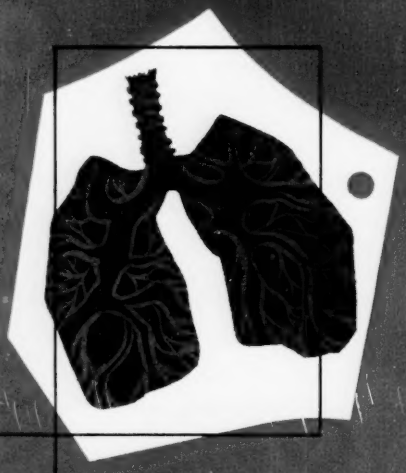

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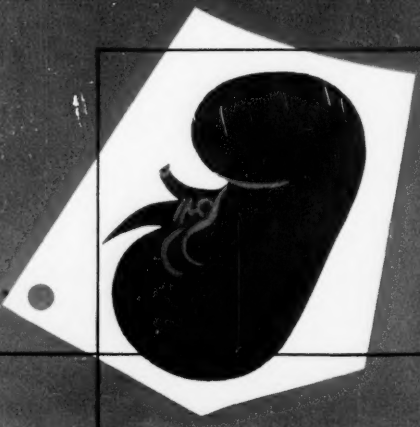

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Diagnostic Criteria of Vascular Headaches

		MIGRAINE	MIGRAINE EQUIVALENTS	MIGRAINE VARIANTS (e.g. Histaminic Cephalgia)	TENSION HEADACHE
BACKGROUND	INCIDENCE	8 to 10 million in U.S.; (ratio of women: men::2.5:1)	As common as migraine	Less common than migraine (mostly in men)	Most common of all headaches
	FAMILIAL HISTORY	Hereditary factor	Hereditary factor	Not significant	Not significant
ONSET OF ATTACK	STAGE OF LIFE	Puberty to menopause	Puberty to menopause	Often after 35	Any
	RATE OF ONSET	Abrupt to gradual	Abrupt to gradual	Abrupt	No pattern
	TIME OF DAY	Usually early morning, diminishing in evening	Variable	Frequent during sleep	Usually at end of day
	DAY OF WEEK, MONTH, ETC.	Most commonly weekends; frequently pre-menstrual and during periods of conflict, tension or stress.	Same as classical migraine	Occur in series, as often as 10 to 15 times a day, often seasonal.	Variable, but usually preceding or following emotional stress or conflict
DESCRIPTIVE QUALITIES OF HEADACHE	DURATION	Minutes to days	Minutes to days	Under 1 hour	No pattern
	NATURE OF HEAD PAIN	Throbbing or pulsating		Constant, boring	Sustained dull or sharp pain
	SITE	Anywhere in head and face—most commonly at right temple, usually unilateral	*SEE NOTE BELOW	Involves eye, neck and often face; unilateral	Most intense in neck, shoulders and occiput—may spread to frontal region—unilateral or bilateral
	TENDERNESS (RESIDUAL)	Near large extra-cranial arteries. Affected region may involve nasal, paranasal, teeth, ear, neck.	Varies according to location	No residual tenderness	Any of head and neck muscles
	EFFECT OF MANUAL PRESSURE	Pressure upon temporal, frontal, post-auricular arteries often reduces intensity	*NOTE Many cases in this classification suffer pain in regions other than the head	Similar to migraine (classical)	Pressure upon tender muscles or regions of tenderness intensifies headache
	EFFECT OF POSITION OF HEAD	Erect position relieves—shaking head aggravates		Pain eased by sitting up and leaning forward	Shaking head reduces intensity by extending muscles
ASSOCIATED SYMPTOMS OF ATTACK	VISUAL DISTURBANCES	Scintillating scotomata; unilateral homonymous hemianopia usually of short duration preceding attack. Photophobia common	Same as classical migraine Photophobia always present in ophthalmic migraine	Photophobia often present	Partial closure of eyes due to muscle spasm. May give impression of faulty vision; Photophobia common
	OTHER OCULAR SYMPTOMS	Injection of conjunctiva and sclera, lacrimation and ptosis of eyelid may occur	Ptosis of eyelid occurs with ophthalmoplegic migraine	Conjunctival injection, lacrimation, nasal stuffiness, discharge	Injection of sclera and conjunctiva; lacrimation may also occur
	VERTIGO AND OTHER SENSORY DISTURBANCES	May be present; paresthesias of hands and face may precede	Paresthesias of hands and face may precede	Infrequent	Infrequent
	MOOD CHANGES	Depression-irritability precede; exaltation follows	Same as classical migraine	No significant changes	Tension, irritability; desire for attention
	GASTRO-INTESTINAL DISTURBANCES	Anorexia, nausea and vomiting at height of attack; flatulence and distension	In gastro-intestinal migraine - flatulence, distension, constipation	Infrequent	Flatulence and distension common especially with severe headache
	CHANGE IN BODY TEMPERATURE	Slight; sometimes unilateral sweating	In some cases up to 104°F	Not significant	Chills and hot flashes without change in temperature
	TREATMENT	Emotional guidance, general improvement in health; Endocrine therapy. Specific for attack: Cafergot	Emotional guidance, general improvement in health; Specific for attack: Cafergot; vaso-constrictor agent		Sedatives

The information presented in this table has been compiled by the Sandoz Scientific and Research Staffs from the following publications:
AYASH, J. J.: Journal Lancet 69: 389 (Nov.) 1949. COHEN, S. G., and CRIEP, L. H.: New England J. Med. 241: 896 (Dec. 8) 1949. FRIEDMAN, A. P.: Am. Pract. 1: 948 (Sept.) 1950. FRIEDMAN, A. P., and BRENNER, C.: Am. Pract. 2: 467 (March) 1948. HANSEL, F. K.: Ann. Allergy 6: 151 (March-April) 1949. HILSINGER, R. L.: Christ Hospital Bulletin 2: 85 (April) 1949. MACNEAL, P. S.: Philadelphia Medicine 44: 1869 (July 9); 1905 (July 16) 1949. MOENCH, L. G.: Headache, Chicago, Year Book Publishers, 1948, chap. 8, 9 and 10. SHEA, J. J.: J. Omaha Mid-West Clin. Soc. 11: 71 (Aug.) 1950. WALSH, F. M.: Journal Lancet 70: 259 (July) 1950. WOLFF, H. G.: Headache and Other Head Pain, New York, Oxford University Press, 1948.

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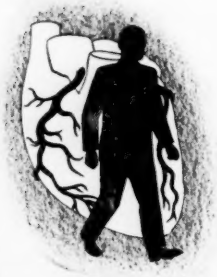

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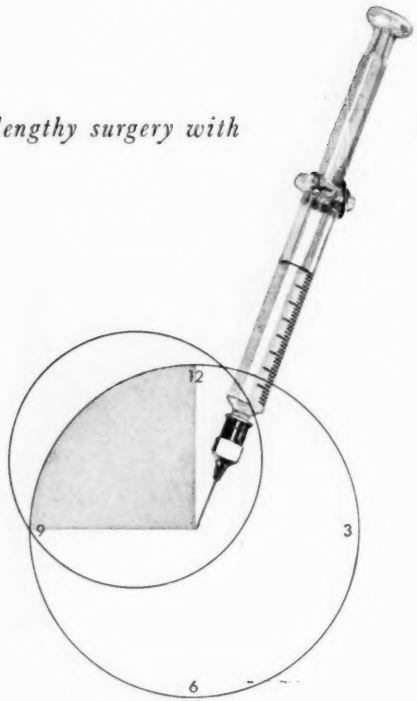

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THE EFFECTS OF ADRENOCORTICOTROPIC HORMONE AND CORTISONE IN THE ADRENOGENITAL SYNDROME ASSOCIATED WITH CONGENITAL ADRENAL HYPERPLASIA: AN ATTEMPT TO EXPLAIN AND CORRECT ITS DISORDERED HORMONAL PATTERN [1, 2]

By FREDERIC C. BARTTER,[3] FULLER ALBRIGHT, ANNE P. FORBES, ALEXANDER LEAF,[4] ELEANOR DEMPSEY, AND EVELYN CARROLL

(From the Department of Medicine of the Harvard Medical School and the Medical Service of the Massachusetts General Hospital, Boston)

(Submitted for publication November 2, 1950; accepted, December 9, 1950)

The adrenogenital syndrome associated with congenital adrenal hyperplasia (1) is in many of its aspects the direct opposite of the other syndrome which may result from adrenal hyperplasia, namely, Cushing's syndrome (2). For example, the former is characterized by rapid growth before epiphyseal closure, and an increase in strength, muscle mass, and skin thickness, whereas the latter is characterized by cessation of growth before epiphyseal closure, and a decrease in strength, muscle mass and skin thickness.

Since the effect of adrenocorticotropic hormone (ACTH) in subjects with normal adrenals is essentially to produce a temporary "Cushing's syndrome medicamentosa" (3–7) it was of interest to observe the effects of ACTH in the adrenogenital syndrome. The first part of this report concerns the effects of ACTH in three female patients with the adrenogenital syndrome.

The results, which were surprising, led to the supposition that cortisone might inhibit the excessive production of androgens in these patients. The second part of this report concerns the effects of cortisone in two female patients with the adrenogenital syndrome.

METHOD

With the exception of the long-term studies which are still in progress (*vide infra*) all studies were carried out on the Metabolic Ward. The diet and fluid intake were kept constant throughout each study. Stools were collected in six day periods, and urines in one to six day pools. Stools and diets were analyzed for N, P, Ca, K, Na, and Mg,[5] and urines for these constituents and for Cl, "reducing steroids," and 17-ketosteroids. Fasting blood for determination of circulating eosinophils, and of serum glucose, Ca, P, Mg,[5] Na, K, Cl, and CO_2 was drawn at appropriate intervals. The following methods were used: Magnesium—Briggs (8); reducing steroids—Talbot and his associates (9) and Heard, Sobel and Venning (10); eosinophil counts— Hills, Forsham and Finch (11), except that the blood was diluted 1:10 instead of 1:20; the remaining determinations—as described elsewhere (12).

CASE HISTORIES

C. O., M.G.H. No. 169, 536, was a 13 year old girl who had been seen first at the age of five because of the presence of pubic hair since the age of two. Growth proceeded rapidly, with markedly advanced bone age, and ceased by the age of nine with epiphyseal closure. Menses had not appeared.

Physical examination revealed an abnormally muscular girl, a height and span both of 57½ inches, a large thyroid cartilage, a deep voice, temporal recession of the hairline, hirsutism of face and body, a large amount of pubic and axillary hair, scant breast tissue, a very large clitoris, absent labia minora, and a common external orifice of urethra and vagina. X-rays revealed no adrenal tumor after air insufflation, and a normal skull. Repeated chemical analyses revealed normal serum glucose, Na, Cl, K, Ca, P, CO_2 content, and alkaline phosphatase. A Cutler-Power-Wilder test revealed a normal four hour chloride excretion. Twenty-four hour urine specimens contained normal amounts of reducing steroids, subnormal amounts (less than 2.8 mouse units) of "cortin" (13), and very large amounts (60 to 70 mg.) of 17-ketosteroids.

An electroencephalogram was read by Dr. John Abbott as follows: "This is an abnormal record for her age. It shows a mild, diffuse, slow-wave cerebral dysrhythmia (many six per second waves). Voltages are slightly higher on the right."

T. McH., M.G.H. No. 586, 528, was a ten year old girl

[1] Reported in part at the Proceedings of American Society for Clinical Investigation, May 1, 1950. (J. Clin. Invest., 1950, **29**, 797.)

[2] The expense of these studies was defrayed by a grant from the Rockefeller Foundation, a grant from Ayerst, McKenna and Harrison, Ltd., and grants from the American Cancer Society. A bed supported by Mr. Edward Mallinckrodt, Jr., on the Metabolic Ward was used for these studies.

[3] Surgeon, U.S.P.H.S., National Heart Institute.

[4] National Research Council Fellow in the Medical Sciences, 1949–1951.

[5] Not done on patient C. O.

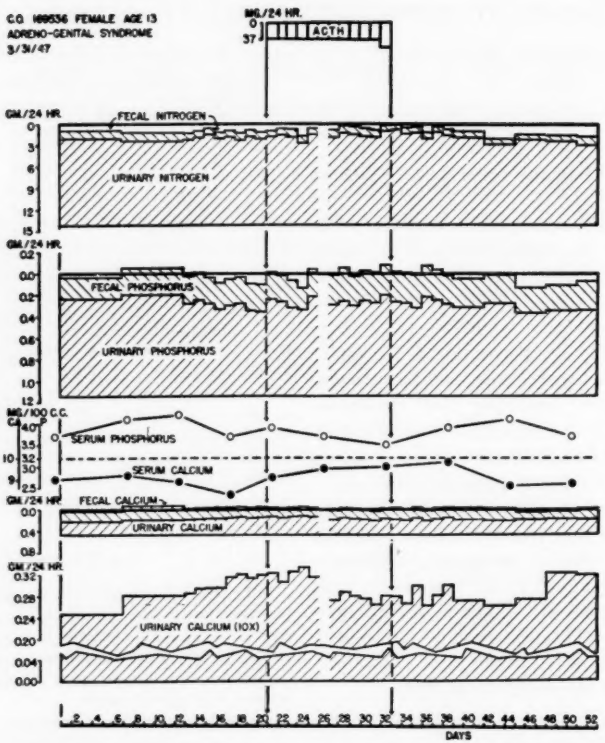

FIG. 1. N, P, AND Ca BALANCES, URINARY Ca, AND SERUM P AND Ca DURING ADMINISTRATION OF ACTH TO C. O., A 13 YEAR OLD FEMALE WITH THE ADRENOGENITAL SYNDROME

Balance data in this and subsequent charts are plotted as follows: There is a horizontal base line; intake is charted downward from this base line; the urinary and fecal excretions are then measured upward from the intake line towards the base line. If the output (fecal and urinary) exceeds the intake, the final level will be above the base line; if it does not, the final level will be below the base line. Thus a negative balance is indicated by a shaded area above the base line, and a positive balance by a clear area below the base line. The scales for N, P, Ca, and K metabolism are so chosen that (for changes in protoplasm and bone) the area representing P balance should equal the sum of the corresponding areas for N and Ca; and that representing K balance should equal that for N.

who was referred to us by Dr. Gertrud Ryersbach. She had been seen first at the age of eight because of the presence of pubic hair since the age of four. Growth proceeded rapidly, with markedly advanced bone age. Menses had not appeared except upon withdrawal of estrin therapy, which had been tried without appreciable effect on the virilism.

Physical examination revealed a well-developed girl, a height and span of 65 and 65¾ inches respectively, a slightly enlarged thyroid cartilage, a voice not deeper than that of a normal adult female, a large amount of pubic and a moderate amount of axillary hair, a moderate amount of breast tissue (estrogen therapy), a very large clitoris, and otherwise normal external genitalia. X-rays revealed a normal skull. Repeated chemical analyses revealed normal serum glucose, Na, Cl, K, Ca, P, CO_2 content, and alkaline phosphatase. A Kepler water test gave a normal result. Twenty-four hour urine specimens contained normal amounts of reducing steroids, and large amounts (14 to 18 mg.) of 17-ketosteroids.

Ten electroencephalograms were read by Dr. John Abbott as follows: "These are abnormal records for her age. They show severe diffuse, slow-wave cerebral dysrhythmia (random waves and paroxysmal runs, notably three per second waves reaching 150 micro-volts, and six per second waves reaching 175 micro-volts). Voltages are markedly higher, and dysrhythmia is more pronounced, on the right. These abnormalities are especially

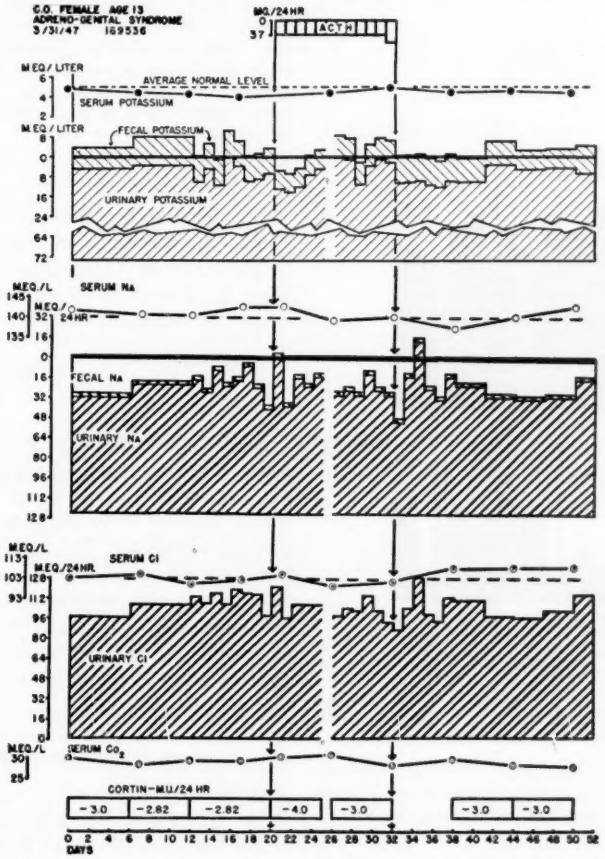

FIG. 2. K AND Na BALANCES, URINARY Cl AND CORTIN, AND SERUM K, Na, Cl AND CO_2 CONTENT IN EXPERIMENT SHOWN IN FIGURE 1

For interpretation of cortin see Forbes, Griswold and Albright (25).

marked in the occipitoparietal region a little to the right of the midline. There are traces of normal alpha activity at eight per second and 50 micro-volts."

On explorations by Doctor Oliver Cope, the internal genitalia were found to be of the normal female structure; ovarian biopsy revealed developing follicles; the right adrenal gland was not grossly abnormal to palpation; and the left adrenal cortex showed hypertrophy on biopsy.

R. H., M.G.H. No. 401, 611, was a 12 year old girl who had been seen first at the age of five because of the presence of an enlarged clitoris since the age of three months and pubic hair since the age of four. Growth proceeded rapidly, with advanced bone age, and ceased by the age of nine with epiphyseal closure. Menses had not appeared, even on withdrawal of estrogen therapy, which had been tried without appreciable effect on the virilism.

Physical examination revealed a well-developed girl, a height and span both of 62 inches, a slightly enlarged thyroid cartilage, a moderately deep voice, slight temporal recession of the hairline, hirsutism of the upper lip and body, large amounts of pubic and axillary hair, a moderate amount of breast tissue (estrogen therapy), a very large clitoris, absent labia minora, and a common external orifice of urethra and vagina. X-rays revealed normally placed kidneys and a normal skull. Repeated chemical analyses revealed normal serum glucose, Na, Cl, K, Ca, P, CO_2 content, and alkaline phosphatase. Twenty-four hour urine specimens contained normal amounts of reducing steroids and large amounts (17 to 25 mg.) of 17-ketosteroids.

Four electroencephalograms were read by Dr. John Abbott as follows: "These are abnormal records for her age. They show a diffuse, slow-wave, cerebral dysrhythmia (four to six per second waves). The abnormality is somewhat more marked on the left. There is some normal alpha activity at around eight to nine per second and 40 micro-volts."

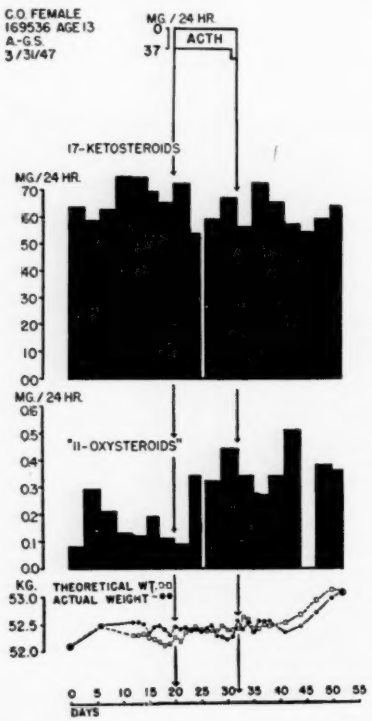

FIG. 3. 17-KETOSTEROIDS AND "11-OXYCORTICOSTEROIDS," ACTUAL WEIGHT, AND THEORETICAL WEIGHT BASED ON N, Na, AND K DURING EXPERIMENT SHOWN IN FIGURE 1

For calculation of theoretical weight see Reifenstein and associates (12). The upper limit of normal for "11-oxycorticosteroids" by this method is 0.44 mg. per 24 hours (26).

RESULTS

The overall design of each metabolic study may be seen by inspection of Figures 1, 4, and 7. Seven courses of therapy were given as shown in Table I. The metabolic effects of the two hormones are best considered separately.

I. ACTH

a) Nitrogen metabolism

ACTH did not produce a significantly negative nitrogen balance in any of the four courses of administration (Figures 1, 4, and 7). Indeed, when a daily dose of 200 mg. was given, *there was actual nitrogen retention.* This observation is contrary to the experience of ourselves (14, 15) and others (3–6) in patients with essentially normal adrenal cortical function. Figure 10 is a scatter diagram contrasting the total six day N excretion of other patients given comparable doses of ACTH with that of these patients.

b) Phosphorus metabolism

When 200 mg. of ACTH were given to patient T. McH. (Figure 4) there was retention of P commensurate with that of N, on the assumption that protoplasm was formed (12). In the other experiments (Figures 1 and 7) ACTH did not affect P balance or serum P.

TABLE I

The dosage and duration of ACTH and cortisone administration and the composition of the diet in the metabolic studies reported

Wt. at start of treatment	Patient	Drug	Daily dose		Units pressor per day†	No. of days	CHO	Fat	Protein	Calories
kg.			*mg.**	*mg.* per kg.*						
52	C. O.	ACTH	37	.71	2.6	12‡	250	103	83	2258
55	T. McH.	ACTH§	84	1.53	4	12	246	61	74	1828
60	T. McH.	ACTH	200	3.33	8	12	388	57	59	2317
47	R. H.	ACTH	84	1.79	4	9	179	57	54	1446
53	T. McH.	Cortisone acetate§	100	1.90		12	246	61	74	1828
45	R. H.	Cortisone acetate	100	2.22		12	179	57	54	1446
43	R. H.	Combined ACTH and cortisone	88 100	2.05 2.32	4	8	179	57	54	1446

* Expressed as equivalents of Armour Standard La 1A.
† The pressor is present in the ACTH as contaminant and was assayed by the Armour Laboratories.
‡ On the 12th day, 55 mg., or 1.06 mg. per kg., were given.
§ Stilbestrol, 5 mg. a day, was administered throughout this experiment.

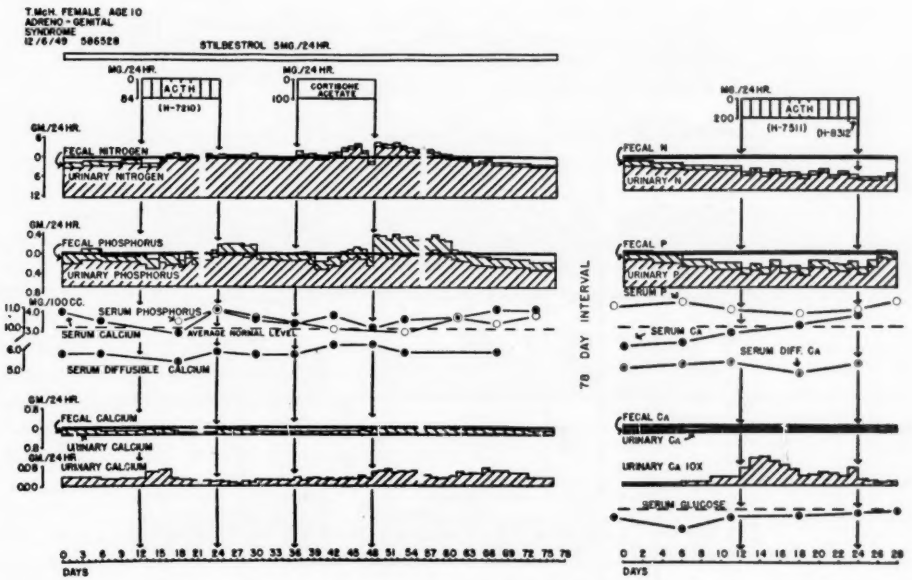

FIG. 4. N, P, AND Ca BALANCES, URINARY Ca, SERUM P, Ca AND DIFFUSIBLE Ca DURING ADMINISTRATION OF ACTH AND CORTISONE TO T. McH., A TEN YEAR OLD FEMALE WITH THE ADRENOGENITAL SYNDROME

The determinations of diffusible Ca in this and the subsequent experiments were done for us by Dr. John Dawson.

c) Calcium metabolism

There was a rise in urinary calcium with ACTH in all experiments save that on C.O. (Figure 1). As discussed elsewhere (7, 16) this effect may be due to the pitressin present in the ACTH as a contaminant (Table I). Comparison of the actual weight curves with the "theoretical" weight curves based on N-plus-K-plus Na (Figures 3, 6, and 9) shows that water was retained without electrolytes during ACTH therapy—presumably a pitressin effect—in the experiments on T. McH. and R. H. Water was not retained in C. O., and she showed no rise in urinary calcium.

d) Potassium metabolism

ACTH produced a slight, unsustained retention of K in all experiments. This is in contrast to our previous experience (7), wherein a transitory rise of urinary K, with negative balance, on the first day of therapy, and a transitory fall, with positive balance, on the first or second day after stopping have been among the most constant effects of ACTH. This is shown in Figure 11, wherein the effect of ACTH on urinary K excretion in 12 experiments on patients with essentially normal adrenal cortical function is contrasted with that in these patients.

e) Sodium and chloride metabolism

In the first experiment on T. McH. and that on C. O., ACTH had no appreciable effect on Na or Cl metabolism (Figures 2 and 5). In the other two experiments, slight loss of Na and Cl occurred (Figures 5 and 8). As discussed elsewhere, the effect of these preparations of ACTH upon Na and Cl appears to be a resultant of the

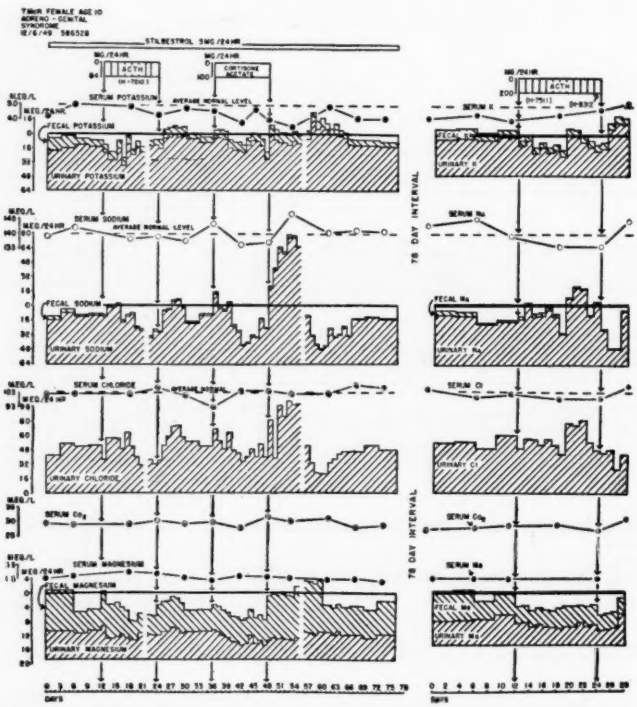

FIG. 5. K, NA AND MG BALANCES, URINARY CL AND SERUM K, NA, CL, CO_2 CONTENT, AND MG DURING THE EXPERIMENT SHOWN IN FIGURE 4

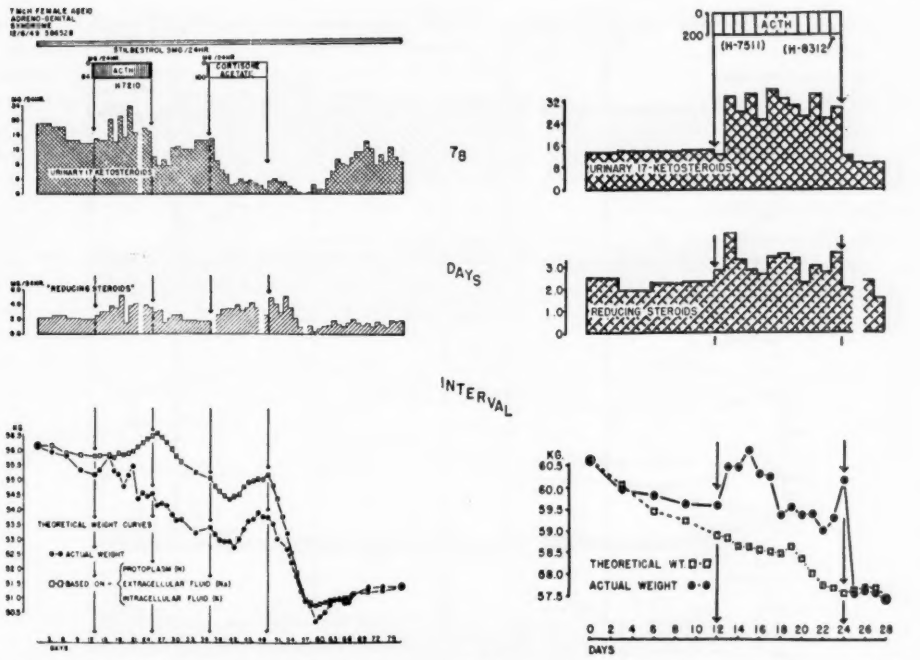

FIG. 6. URINARY 17-KETOSTEROIDS AND "REDUCING STEROIDS," ACTUAL AND THEORETICAL WEIGHT BASED ON N, NA, AND K DURING EXPERIMENT SHOWN IN FIGURE 4

The upper limit of normal for reducing steroids by this method is 5 mg. per 24 hours.

sodium-retaining properties of the adrenal steroids and the sodium diuretic properties of contaminating posterior pituitary substances (7, 17). Similar doses of these preparations of ACTH produce sodium retention in patients with essentially normal adrenal cortical function, in spite of the presence of pitressin in quantities equal to or greater than those given here. Thus there is no evidence in these patients that sodium-retaining steroids were released in response to ACTH.

Lewis and Wilkins (18) reported sodium loss following administration of ACTH for a single day to two patients with the adrenogenital syndrome. The amount of contaminating pitressin their patients received was relatively high. It should be noted, however, that sodium retention very frequently fails to occur during the first 24 hours of ACTH therapy to patients who later show marked retention (4–7).

f) Magnesium metabolism

Mg balances were measured during three of the four studies with ACTH. When a daily dose of 200 mg. was given (Figure 5) urinary Mg fell. In the other experiments (Figures 5 and 8) no consistent effect on Mg metabolism was noted.

g) 17-Ketosteroids

In all subjects the 17-ketosteroid excretion was very high (19) for the age of the patient. In patient C. O., ACTH produced no further rise in 17-ketosteroid excretion (Figure 3); in T. McH., a questionable rise was produced with a dosage of 84 mg., and a marked rise with 200 mg. a day (Figure 6). In patient R. H. a marked rise was produced with 84 mg. a day (Figure 9).

h) Reducing steroids ("11 oxysteroids")

ACTH did not produce a significant rise in reducing steroids in any of the experiments (Figures

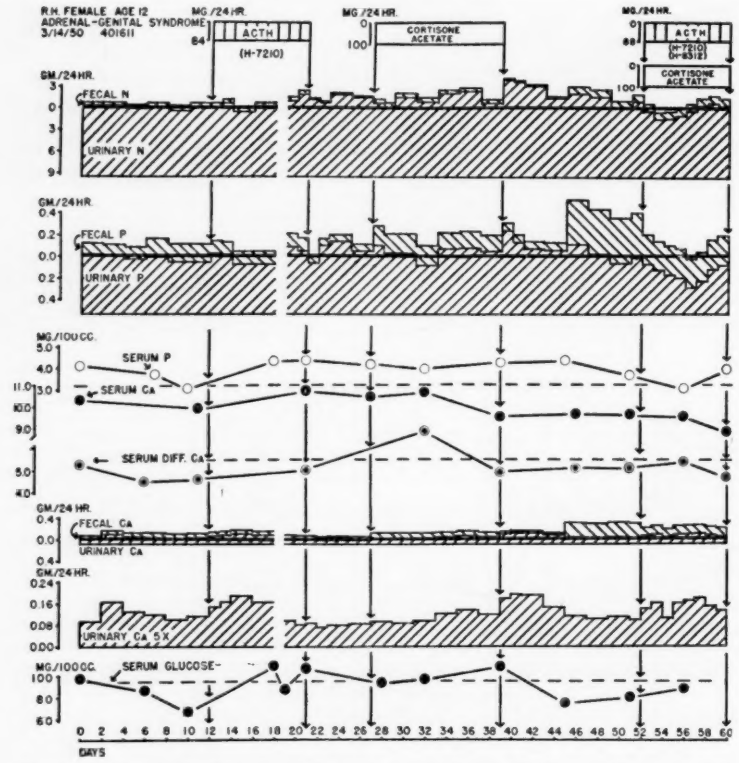

FIG. 7. N, P, AND Ca BALANCES, URINARY Ca AND SERUM P, Ca, AND DIFFUSIBLE Ca AND GLUCOSE DURING ADMINISTRATION OF ACTH AND CORTISONE TO R. H., A 12 YEAR OLD FEMALE WITH THE ADRENOGENITAL SYNDROME

3, 6, and 9). This result is in striking contrast to the elevations of urinary reducing steroids of two to ten times the normal values which have been consistently reported following similar doses of ACTH (3, 4, 6, 15).

i) Circulating eosinophils

The initial counts of fasting eosinophils were high in the two patients (T. McH. and R. H.) in whom they were done. With ACTH (Figure 12) there was a transient fall, followed by a rise to or above the initial values, despite continued ACTH therapy. This observation is in contrast to the well-documented observation (3–6) that the circulating eosinophils fall to approximately zero and remain there with comparable doses of ACTH in patients with essentially normal adreno-cortical function.

j) Glycosuria

No patient developed glycosuria at any time during therapy.

Summary of results of ACTH therapy

In many respects, these patients responded abnormally to ACTH. The failure to increase significantly the excretion of reducing steroids, to develop a negative N balance, or to show a large and sustained fall of circulating eosinophils is an indication that the output of "carbohydrate-active"

steroids[6] ("sugar" hormone) following ACTH is markedly subnormal. The failure to develop transient K diuresis is an indication that the output of either "carbohydrate-active" or "salt-retaining" steroids or both is subnormal—if, indeed, these are separate hormones (20). The failure to develop Na and Cl retention is an indication that the output of "salt-retaining" steroids is similarly impaired.

In one respect these patients responded normally to ACTH. In spite of high initial levels, the urinary 17-ketosteroid excretion rose in all experiments except one, that on C. O. In this experiment the initial level was highest, and the lowest dose of ACTH was given. The urinary 17-ketosteroids are presumably (2) an index of the adrenal androgen which is responsible for the virilism in these patients, and the initially high values are of course a cardinal feature of the disease.

These findings suggested two metabolic disorders in the pathogenesis of this condition: diminished ability of the adrenal cortex to respond to ACTH with an increased output of carbohydrate-active and salt-retaining steroids, and an increased production of ACTH (*vide infra*) resulting in the abnormally high production of adrenal androgen (see also Figure 13).

It was then postulated that a carbohydrate-active adrenal cortical hormone such as cortisone should depress the production of ACTH and thus diminish adrenal androgen production. Conse-

[6] Probably these consist of Compound F, Compound E, or closely related steroids.

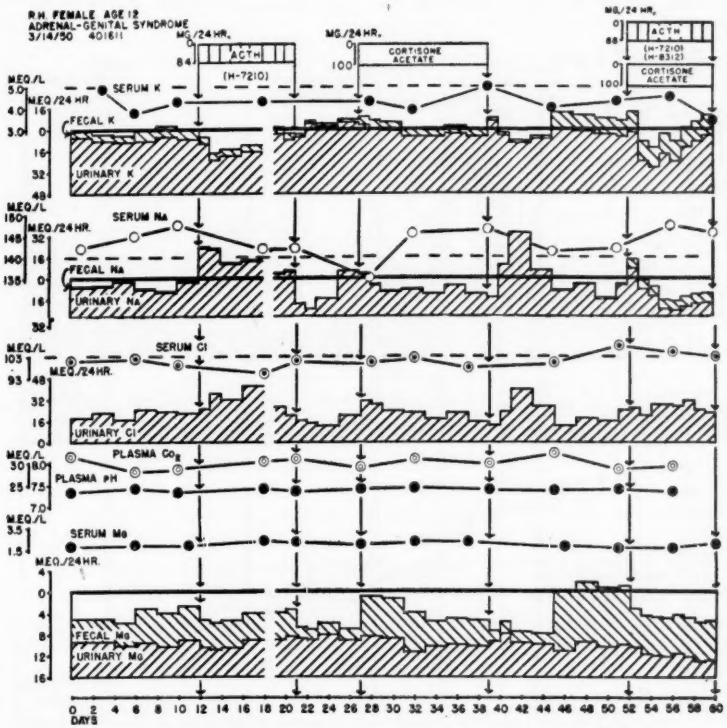

FIG. 8. K, Na, and Mg Balances, Urinary Cl, Serum K, Na, Cl, CO_2 Content, and Mg, and Plasma pH during the Experiment Shown in Figure 7

quently, cortisone acetate was administered in doses of 100 mg. a day for 12 days to patients T. McH. and R. H.

II. Cortisone

The metabolic effects of cortisone are shown in Figures 4–9. The changes in P, Ca, K, Na, and Cl metabolism and in excretion of reducing steroids are not remarkable, and do not differ essentially from those previously reported (6) following the use of cortisone acetate at similar dosages. There was a slight fall of urinary Mg in both patients.

Cortisone produced a rise in urinary N in both patients, resulting in a markedly negative N balance in T. McH. (Figure 4) and increasing the already existing negative N balance in R. H. (Figure 7). This result is contrary to those of Sprague and associates (6), Clark, Ropes and Bauer (21), and ourselves (22), following the use of cortisone at this dosage in patients with other syndromes. Sprague and his co-workers state, "As a rule, the augmentation of nitrogen excretion was slight or absent when the dose of cortisone was 100 mg. daily."

The second striking result of cortisone therapy was the marked progressive fall in 17-ketosteroid excretion in both patients (Figures 6 and 9). The urinary 17-ketosteroids remained low for eight (R. H.) and 12 (T. McH.) days, then rose gradually to the pre-injection levels. A similar depression of 17-ketosteroids with cortisone in a patient with the adrenogenital syndrome has been reported by Wilkins and his colleagues (23).

III. ACTH and Cortisone

As stated above, we had believed, *a priori*, that the effect of cortisone would be to lower the uri-

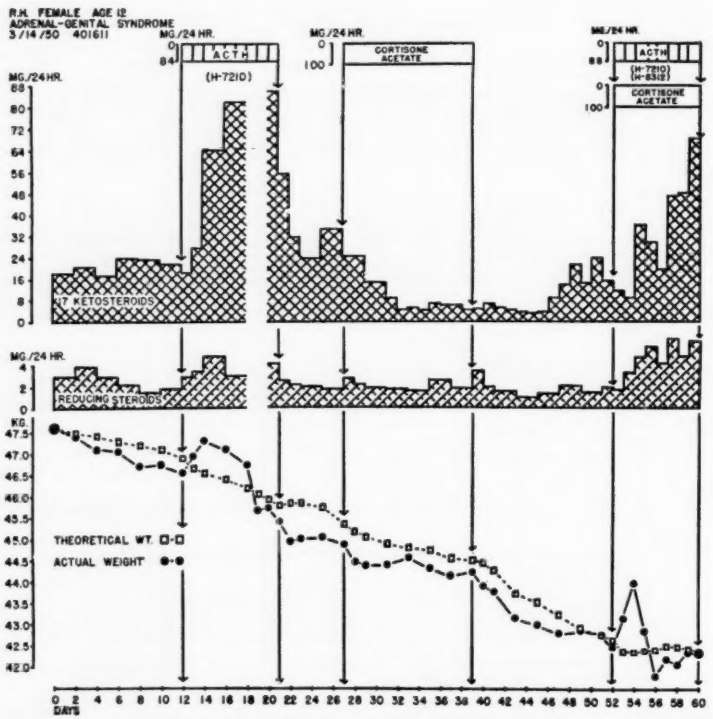

FIG. 9. URINARY 17-KETOSTEROIDS, "REDUCING STEROIDS," ACTUAL WEIGHT, AND THEORETICAL WEIGHT BASED ON N, NA, AND K DURING EXPERIMENT SHOWN IN FIGURE 7

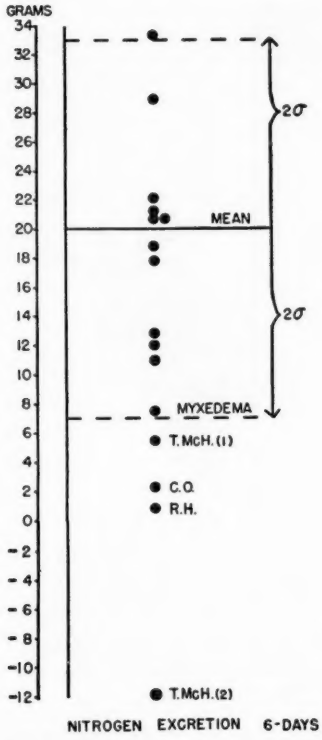

FIG. 10. THE TOTAL N EXCRETION DURING THE FIRST SIX DAYS OF ACTH ADMINISTRATION TO THE THREE PATIENTS REPORTED, CONTRASTED WITH THAT IN 11 COMPARABLE EXPERIMENTS ON PATIENTS WITH ESSENTIALLY NORMAL ADRENAL CORTICAL FUNCTION, AND ONE ON A PATIENT WITH MYXEDEMA

Nitrogen excretions were calculated as deviations from the average of control values. Note decreased N excretion in patients with the adrenogenital syndrome, and actual N retention with 200 mg. of ACTH in T. McH. (*2*).

nary 17-ketosteroid excretion by depressing pituitary ACTH production, and not by direct action on the adrenal gland. To provide further evidence on this point we decided to administer ACTH and cortisone together to patient R. H. If cortisone has a direct action on the adrenal cortex it should decrease the response to administered ACTH. The results show that the rise in 17-ketosteroid excretion resulting from ACTH together with cortisone (Figure 9, days 53 through 60) did not differ essentially from that resulting from ACTH alone (Figure 9, days 13 through 20). Thus they do not show direct action of cortisone on the adrenal cortex or on its response to ACTH in this patient. Hence, they support the view that cortisone acts by inhibiting endogenous ACTH.

DISCUSSION

a) Nitrogen metabolism

Consideration of the adrenogenital syndrome, which may be associated with either hyperplasia or tumor of the adrenal cortex, leaves little doubt that the adrenal cortex may produce steroids which have virilizing properties. There is considerable circumstantial evidence (*2*) that the measurement of urinary 17-ketosteroids provides an index of the quantities of such steroids being produced. Further, there is clinical evidence (*2*) that these steroids may have somatotropic, nitrogen-retaining, as well as virilizing properties.

The present study provides two pieces of indirect evidence in support of the latter point. In the first place, when T. McH. received 200 mg. of ACTH a day, there was a marked rise in 17-ketosteroid excretion without a significant rise in urinary reducing steroids. Under these circumstances, nitrogen retention was increased.[7] Nitrogen retention with ACTH has not heretofore been described, presumably because nitrogen loss with "sugar" hormone outweighs any nitrogen retention due to somatotropic hormones. In the second place, when T. McH. and R. H. received 100 mg. of cortisone a day there was a marked fall in 17-ketosteroid excretion. Under these circumstances, more loss of N was produced than has been observed following this dosage[8] of cortisone. Thus in the first instance, with a rise of adrenal androgen unopposed by adrenal "sugar" hormone there was N retention, whereas in the second instance, with a fall of adrenal androgen there was an abnormally great N loss following exogenous "sugar" hormone.

[7] N retention did not occur in R. H. in spite of a greater rise in 17-ketosteroid excretion. This may be a result of qualitative differences in the precursors of the 17-ketosteroids in these two patients (13, 24).

[8] Either on an absolute or a mg. per kg. basis.

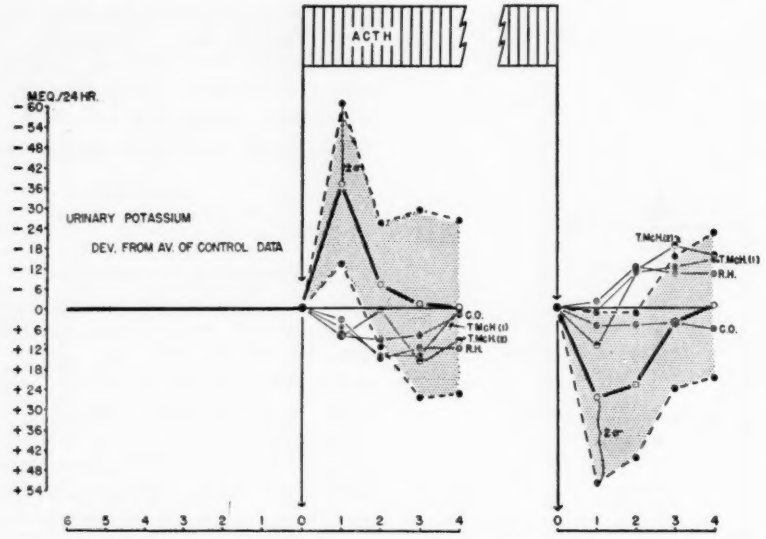

FIG. 11. THE URINARY K EXCRETION DURING THE FIRST FOUR DAYS OF, AND THE FIRST FOUR DAYS AFTER, ADMINISTRATION OF ACTH TO THE PATIENTS REPORTED CONTRASTED WITH THAT IN 11 COMPARABLE EXPERIMENTS ON PATIENTS WITH ESSENTIALLY NORMAL ADRENAL CORTICAL FUNCTION

The shaded area is that covered by two standard deviations above and below the mean normal curve (heavy black line). Note that patients with the adrenogenital syndrome did not show the K diuresis on starting nor the K retention on stopping ACTH.

b) Clinical implications

During these short metabolic studies, no clinically apparent change in these patients was expected or found. Subsequent to these studies, T. McH. has received continuously 100 mg. of cortisone twice a week (total duration, five months to date of writing) and R. H. 50 mg. twice a week (total duration, six months to date of writing).

In neither patient has this therapy produced a fall in the number of circulating eosinophils; in both, it has maintained the 17-ketosteroid excretion at a level approximately normal (19) for the patient's age and sex. T. McH. has begun to have regular periods with cramps. It is too early to say whether further virilism can be prevented by this form of therapy.

c) Interpretation of the pathological physiology

There are a number of possible explanations for the findings reported here. One such explanation is shown graphically in Figure 13. It is postulated that adrenal androgen inhibits pituitary ACTH production relatively weakly, whereas "sugar" hormones inhibit ACTH relatively strongly. It is suggested that the primary defect in this syndrome is the decreased production of "sugar" hormone by the adrenal cortex in response to ACTH. One then has the following sequence of events:

1) lack of "sugar" hormone,
2) failure of normal inhibition of ACTH,
3) increased output of ACTH, and
4) resulting increase of adrenal androgen.

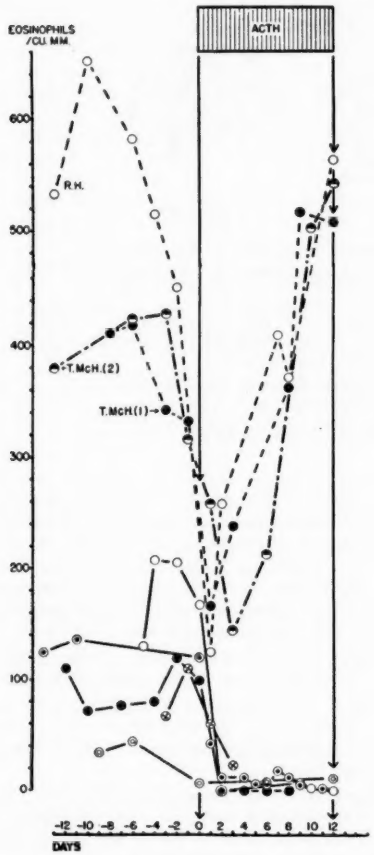

FIG. 12. THE EFFECT OF ACTH ON THE CIRCULATING EOSINOPHILS IN THREE OF THE EXPERIMENTS REPORTED (BROKEN LINES) CONTRASTED WITH THAT IN FIVE COMPARABLE EXPERIMENTS ON PATIENTS WITH ESSENTIALLY NORMAL ADRENAL CORTICAL FUNCTION (SOLID LINES)

Note the high initial level, and the comparatively slight and transitory fall in the patients with the adrenogenital syndrome.

This schema does not require that the patients show an absolute deficit of "sugar" hormone, since the increased production of ACTH may completely compensate for the inherent insensitivity to ACTH.

That there is an increased production of ACTH in this syndrome has not been established. Dr. A. Albert (27) tested the serum of T. McH. and could not demonstrate the presence of ACTH. He did, however, demonstrate the presence of increased amounts of ACTH in the serum of one adult patient from this clinic with a classical case of the adrenogenital syndrome.

SUMMARY AND CONCLUSIONS

1. Four courses of ACTH were administered to three female patients with the adrenogenital syndrome resulting from congenital adrenal hyperplasia. The dosage ranged from .71 to 3.33 mg. per day per kg. body weight, the duration from nine to 12 days. Studies included urinary 17-ketosteroids, reducing steroids, glucose, and chloride, balances of N, P, Ca, K, Mg, and Na, and measurements of circulating eosinophils, serum P, Ca, K, Mg, Cl, CO_2 and glucose.

2. ACTH produced a rise in urinary 17-ketosteroids in three of the four courses, but none of its other usual metabolic effects. With the highest dosage of ACTH there was actual nitrogen retention.

3. Cortisone acetate was administered to two of these patients on the same regimen. The dosages were 1.90 and 2.22 mg. per day per kg. body weight, the duration 12 days.

4. Cortisone produced a fall to normal and even below normal in the elevated urinary 17-ketosteroid excretion, and a negative N balance. The latter is exceptional at the dosages employed.

5. ACTH (2.05 mg. per kg. per day) and cortisone (2.32 mg. per kg. per day) were administered together to one of these patients for eight days. The resulting rise in urinary 17-ketosteroids was essentially the same as that produced by ACTH alone.

6. Cortisone acetate is being administered continuously to two of these patients at dosages of 100 and 200 mg. a week. At this dosage the urinary 17-ketosteroids remain normal and the circulating

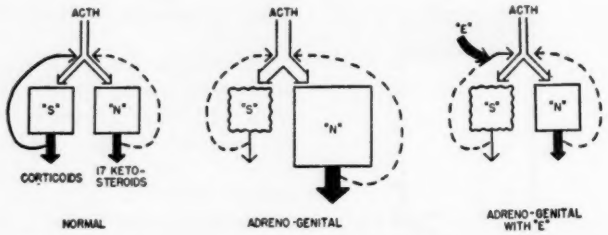

FIG. 13. SCHEMA ILLUSTRATING A POSSIBLE EXPLANATION FOR THE PATHOLOGICAL PHYSIOLOGY OF THE ADRENOGENITAL SYNDROME AND THE EFFECT THEREON OF COMPOUND E

The square figures represent the adrenal cortex. "S" refers to carbohydrate-active ("sugar") hormones, precursors of urinary corticoids; "N" refers to "adrenal androgens," precursors of urinary 17-ketosteroids.

It is postulated that "S" hormones inhibit ACTH effectively (solid arrows directed backwards toward ACTH), whereas "N" hormones do not (broken arrows directed backwards toward ACTH). For further discussion see text.

eosinophils are not depressed. One patient is having monthly periods with cramps.

7. The above findings suggest that this syndrome involves a primary defect of the adrenal cortex, and a secondary increase of pituitary ACTH production.

8. In all patients the electroencephalograms showed asymmetry, elevation of voltages, and abnormal slow waves. This finding is unexplained.

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ERRATUM

In Table VII of the article entitled "Acetylation Studies in Human Subjects with Metabolic Disorders," by H. Gershberg and W. J. Kuhl, Jr., in the December 1950 issue (pp. 1625–1632), the values for both total and free serum cholesterol are *mgm. per cent* rather than as erroneously given.

THE LABILE FACTOR OF PROTHROMBIN CONVERSION: ITS CONSUMPTION UNDER NORMAL AND PATHOLOGICAL CONDITIONS AFFECTING BLOOD COAGULATION [1, 2]

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INTRODUCTION

The rapid conversion of prothrombin to thrombin by thromboplastin plus calcium requires a plasma component called "Labile Factor" (2, 3), which is probably identical with plasma Ac-globulin and Factor V (4, 5), and with the prothrombin accelerator of Fantl and Nance (6, 7).[3] Deterioration of this substance as plasma ages produces an increase in the prothrombin time (2, 3, 8) although the concentration of prothrombin remains essentially unchanged (9, 10). This defect can be rectified by the addition of fresh plasma devoid of prothrombin as well as of fibrinogen (11, 8).

Additional information regarding Labile Factor (L.F.) is desirable for several reasons. Its precise role in prothrombin conversion is obscure, and hemorrhage referable to deficiency of this entity or of a similar substance has been described (12–14). Furthermore, as will become evident, the concentration of L.F. must receive due consideration in procedures which measure prothrombin.

In certain hemorrhagic diseases the conversion of prothrombin to thrombin is reduced or retarded (15, 16). The "prothrombin consumption test," which demonstrates this defect, promises to become important in the investigation and diagnosis of such disturbances. In order to compute the consumption of prothrombin during coagulation, the precise amount of prothrombin in both serum and its parent plasma must be known. Obviously changes in the concentration of L.F. in the transition from plasma to serum may influence considerably the velocity of prothrombin conversion in serum and, therefore, the determination of serum prothrombin by the one-stage procedure, since the prothrombin times may reflect the concentration of L.F. as well as that of prothrombin itself. Under such circumstances the computation of prothrombin consumption from differences between plasma and serum prothrombic activities may be erroneous.

Whereas the concentration of L.F. has been determined in the *plasma* of man and other species (17), little is known regarding its level in *sèrum*. This information is presented in the present report. Also included is a method proposed for the determination of serum L.F. The data indicate that during and after coagulation changes in this clotting factor occur which are considerably influenced by conditions, pathological or induced, which affect clotting.

METHODS

Prothrombin determination: The prothrombic activity of oxalated plasma (one volume of 0.1 M sodium oxalate to nine volumes of blood) was determined on undiluted plasma by the orthodox one-stage procedure (18), and on diluted plasma by the modification (19) in which the test plasma is diluted with fresh normal oxalated pooled plasma rendered prothrombin-free by adsorption with $BaSO_4$.[4] Prothrombin levels were calculated by interpolating the observed prothrombin times on standardization curves obtained on normal plasma similarly diluted. Serum prothrombin was measured similarly and also

[1] Supported by a grant from The Commonwealth Fund.

[2] Presented in part before the Federation of American Societies for Experimental Biology, April, 1950 (1).

[3] It should be pointed out that most investigators feel that Labile Factor, Factor V, plasma Ac-globulin, and the factor of Fantl and Nance are identical. Thus Quick and Stefanini (31) use the terms interchangeably to designate one entity. Seegers and his associates (10) are inclined to agree that Ac-globulin is the same as L.F. and Factor V (30, 40). We (24) as well as Flynn and his coworkers (41) hold the same view. Definite proof regarding the identity of all these moieties, however, is still lacking.

[4] Plasma so treated is hereafter referred to as "$BaSO_4$ plasma."

by the modified two-stage method of Ware and Seegers (20).

Labile factor: Pooled oxalated plasma from normal subjects was stored at 4–5°C for variable intervals during which its prothrombin time increased progressively. L.F. activities of test plasma or sera were measured by their restorative effects on the elevated prothrombin time of these aged plasmas in which the factor had largely deteriorated (8, 17). The restorative effect was determined by adding up to one part of plasma or serum (whole or diluted with physiological saline) to four parts of the aged plasma, and by observing the prothrombin times of the mixtures. The values herein reported are the averages of several observations on each mixture. In ranges below 45 seconds the prothrombin times were always duplicable within two seconds; between 45 and 60 seconds, within four seconds.

Except where otherwise indicated, the serum was separated one hour after venous blood was allowed to clot in a glass tube at room temperature. The clot was rimmed and centrifuged, and the serum was removed and incubated at 37°C for 15 minutes in order to inactivate thrombin. The time interval between incubation of the serum and determination of its activity was noted. Experiments were run on non-oxalated as well as oxalated serum (1 oxalate to 4 serum). In some instances citrate (2.5 per cent sodium citrate) was used instead of oxalate. Also studied were sera which were subjected to adsorption with $BaSO_4$ for 10 minutes at 37°C. Controls for the latter experiments were incubated for the same interval without $BaSO_4$.

Commercial thromboplastin (Difco) and beef lung thromboplastin were used in the one- and two-stage methods, respectively.

RESULTS

The experiments reported below are typical of a series of experiments performed under the same conditions.

Comparative effect of normal serum and plasma on prothrombic activity of aged plasma

Oxalated fresh plasma has a profound restorative effect on the retarded prothrombin conversion of stored plasma (Figure 1). This is not due to the prothrombin in the fresh plasma, since removal of the prothrombin by $BaSO_4$ adsorption does not significantly decrease its restorative activity (Figure 1, Table I). There appears to be remarkably little variation in the L.F. activity of plasma from different normal subjects (Table I). These observations confirm earlier reports (8, 11, 21).

Non-oxalated normal serum tested 75 minutes after coagulation, has as much, and often more, restorative activity than the parent plasma tested simultaneously (Table II). On the other hand,

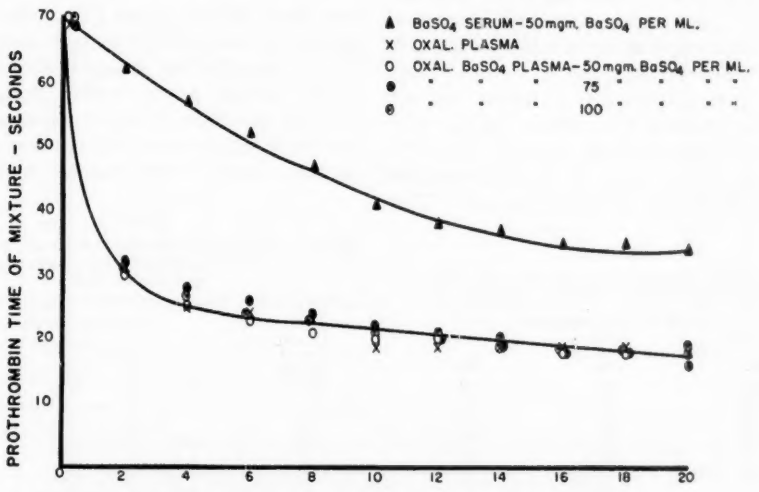

FIG. 1. RESTORATIVE ACTIVITY OF FRESH NORMAL PLASMA, $BaSO_4$ NORMAL PLASMA, AND $BaSO_4$ NORMAL SERUM ON RETARDED PROTHROMBIN CONVERSION OF AGED PLASMA (SIX WEEKS AT 4°C)

TABLE I

Variation of L.F. in fresh normal oxalated plasma before and after adsorption with $BaSO_4$

Subject.....	Prothrombin time (*sec.*) of plasma mixture							
	1		2		3		4	
Per cent fresh plasma in fresh plasma-aged plasma mixture	Whole plasma	$BaSO_4$ plasma	Whole plasma	$BaSO_4$ plasma	Whole plasma	$BaSO_4$ plasma	Whole plasma	$BaSO_4$ plasma
0	87	87	87	87	87	87	87	87
1	43	46	42	46	41	42	40	44
2	36	37	35	36	34	37	35	35
4	29	29	28	30	27	30	27	30
6	27	28	24	27	25	29	26	28
10	23	26	23	24	21	25	21	24
20	19	21	20	22	20	21	19	21

$BaSO_4$ serum is considerably less potent than the control serum (Table III). From interpolation of these data on the curve in Figure 1, it is evident that $BaSO_4$ removes approximately 80 per cent or more of the restorative activity from serum whereas it does not do so from plasma.

Serum has certain clot-promoting properties not demonstrable in plasma (22). This is due to the serum prothrombin conversion accelerator (spca) which arises during coagulation and which in the presence of L.F. accelerates prothrombin conversion (22–24). Accordingly, the accelerating effect of serum on the prothrombin conversion in aged plasma might be due to both spca and L.F.

$BaSO_4$ removes little, if any, L. F. from plasma (8, 21). It is assumed that it likewise does not remove this substance from serum. On the other hand, spca is quantitatively adsorbed (22, 24). Accordingly, $BaSO_4$ serum is devoid of spca but should retain its full complement of L.F. That $BaSO_4$ serum has less restorative ability than $BaSO_4$ plasma indicates, therefore, that the L.F. of normal serum is far less than that of plasma.

Effect of anti-coagulants upon restorative property of normal serum

L.F. is more stable in citrated than in oxalated plasma (8, 25). It is also more stable in native plasma (containing calcium) but not in plasma rendered incoagulable by decalcifying with ion-exchange resins (26). Experiments were performed in order to see whether the L.F. in serum is similarly affected (Table IV). The data indicate that the restorative property of oxalated serum is more labile than that of non-oxalated serum.

The question arises whether oxalate affects L.F., not adsorbable by $BaSO_4$, or some other serum factor such as spca. Experiments performed on non-oxalated versus oxalated $BaSO_4$ serum

TABLE II

Restorative activity of normal plasma and corresponding sera on retarded prothrombin conversion of aged plasma*

	Proth-T† (*sec.*) of mixture‡	
Sal.	Fresh oxal. plas.	Ser.
113	18	19
66	18	17
33	21	17
66	17	18
37	22	16
45	18	16

* All sera separated one hour after clotting at room temperature in glass; both sera and plasma incubated at 37°C for additional 15 minutes and then tested.

† Prothrombin time.

‡ Mixture comprises 0.4 ml. of aged plasma plus 0.1 ml. of material indicated.

TABLE III

Effect of $BaSO_4$ adsorption on restorative activity of normal sera (S) on prothrombin conversion of aged plasma (A.P.)*

Interval between clotting and test	Proth-T (*sec.*) of mixture		
	A.P.	A.P. plus S	A.P. plus $BaSO_4$ S†
hrs.			
3½	33	19	31
2	45	21	31
3½	37	21	34
2	48	21	32
1½	66	22	42

* Non-oxalated sera, separated one hour after blood was shed. One part serum added to four parts A.P.

† Sera adsorbed with 50 mgm. $BaSO_4$ per ml.

TABLE IV

Deterioration of serum L.F. in presence of oxalate and citrate**

Prothrombin time (sec.)

	75 min. after coag.			195 min. after coag.		
	I	II	III	I	II	III
Aged plas.	37	37	37	37	37	37
Aged plas. plus:†						
Ser.	16	15	14	21	17	18
Cit. ser.	17	18	18	21	24	22
Oxal. ser.	18	19	19	28	40	38

* Oxalate and citrate added to normal serum 60 min. after coagulation.

† 0.1 ml. serum added to 0.4 ml. of aged plasma.

(Table V) indicate that the latter, also, was more labile than its non-oxalated counterpart. On the basis of the assumption that $BaSO_4$ does not remove L.F. from serum, it is concluded that oxalate accelerates deterioration of this clotting component.

The restorative activity seems more stable in citrate than in oxalate but not as stable as in native serum (Table IV). It appears, therefore, that L.F. deteriorates more rapidly in serum containing oxalate than in native or citrated serum. The same is observed in plasma (8, 17, 25, 26).

TABLE V

Effect of oxalate on L.F. activity of normal serum (S)

Aged plasma plus:*	Proth-T (sec.)
Saline	61
Fresh S	17
Fresh $BaSO_4$ S	31
Fresh oxal. S	19
Fresh oxal. $BaSO_4$ S	38
S kept 3 hrs., room temp.	20
$BaSO_4$ S kept 3 hrs., room temp.	42
Oxal. S kept 3 hrs., room temp.	32
Oxal. $BaSO_4$ S kept 3 hrs., room temp.	55

Sera separated one hour after clotting.
$BaSO_4$ sera prepared with 100 mgm. $BaSO_4$ per ml.

* One part material added to four aged plasma (60 days at 4–5°C).

Consumption of L.F. and prothrombin during normal coagulation

That L.F. disappears from blood during coagulation is clearly established. Its rate of disappearance was studied in relation to the rate of prothrombin consumption. Aliquots of freshly drawn blood were allowed to clot at 37°C in separate dry test tubes. At specified intervals oxalate was added, the mixture was stirred with a glass rod and immediately centrifuged at 2000 r.p.m. for 10 minutes. The sera were separated and subjected to determination of residual prothrombic activity

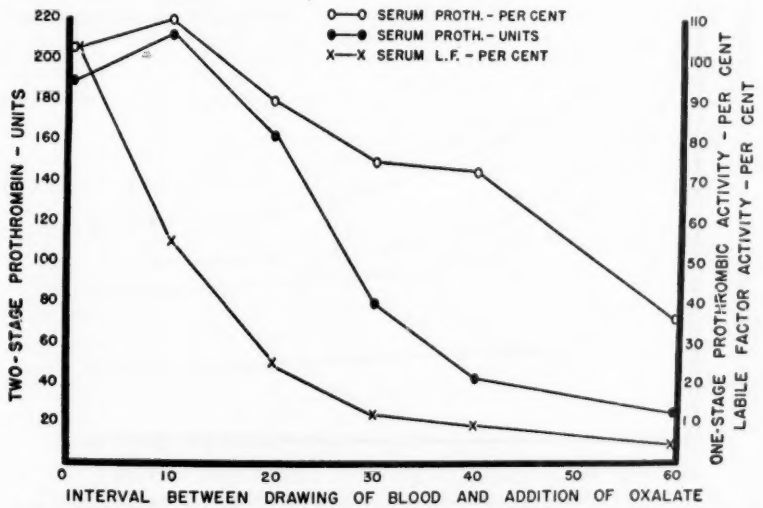

FIG. 2. LABILE FACTOR, AND ONE-STAGE AND TWO-STAGE PROTHROMBIC ACTIVITIES OF "SERUM" DURING COAGULATION OF NORMAL BLOOD

Aged plasma on which L. F. activities were tested had a prothrombin time of 65 sec.

TABLE VI

Effect of thromboplastin (TPL) supplement to clotting blood on L.F. in normal serum (S) (non-oxalated)

Aged plasma plus:	Proth-T (sec.) Exp't 1*	Exp't 2†
Saline	47	63
S	25	33
$BaSO_4$ S	30	44
TPL S	34	47
TPL $BaSO_4$ S	39	64

$BaSO_4$ S prepared with 25 mgm. $BaSO_4$ per ml. TPL S obtained from blood (nine vol.) added to TPL solution (one vol.) prepared as for prothrombin determination. Identical results were obtained when the ratio was 19 to 1.

* One volume of material to four of aged plasma.
† One volume of material to one of aged plasma.

by both one- and two-stage methods. Aliquots of the serum were also adsorbed with $BaSO_4$ (50 mgm. per ml.) and were then tested for L.F. activity. It is evident (Figure 2) that both L.F. and prothrombin disappear during the coagulation process. Whereas L.F. began to decrease promptly after the blood was shed, the two-stage prothrombin values showed no significant change within the first 10 minutes. Within 40 minutes most of both clotting factors had disappeared. There was considerable discrepancy between the one- and two-stage prothrombin values. This is not surprising since one-stage prothrombin times of serum reflect the concentration not only of unconsumed prothrombin but also of residual L.F. together with evolved spca.

It is of interest that within the first 10 minutes, about one-half of the L.F. had disappeared, before any detectable (by the methods used) decrease in prothrombin. Although this must be significant in the role of L.F. in prothrombin conversion, the evidence is too meager to permit any interpretation.

Effect of thromboplastin on concentration of L.F. in serum

Since thromboplastin added to normal blood yields serum which is always devoid of prothrombin in contrast to normal sera which often show small amounts, it was of interest to study the L.F. content of such sera. Sera from thromboplastin-supplemented blood were far less able to restore prothrombic activity to aged plasma than sera from spontaneously clotted blood (Table VI). This

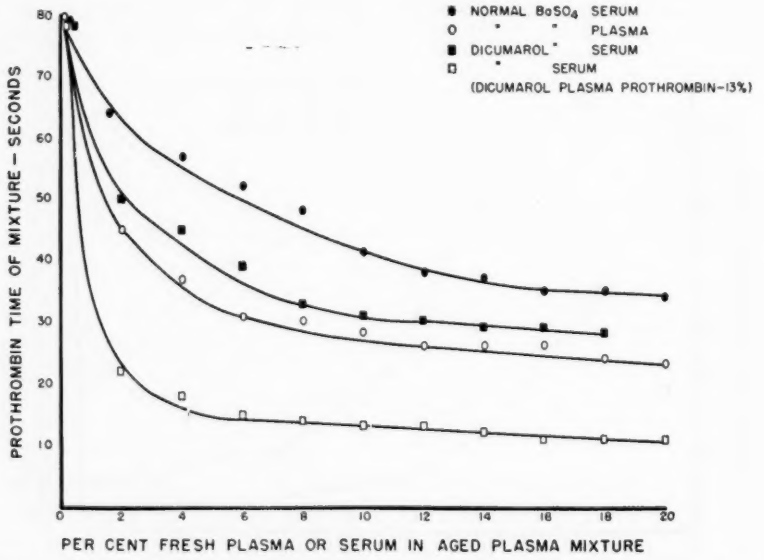

FIG. 3. RESTORATIVE ACTIVITY OF "DICUMAROL" SERUM ON RETARDED PROTHROMBIN CONVERSION OF AGED NORMAL PLASMA

Serum was non-oxalated, and was tested 1½ hours after the blood was obtained. Adsorption with $BaSO_4$ was carried out with 50 mgm. per ml.

difference, evident also in the $BaSO_4$ adsorbed sera, suggests that more L.F. is consumed when coagulation and prothrombin conversion are accelerated by thromboplastin.

L.F. in serum from dicumarolized blood [5]

The purpose of these experiments was to study L.F. depletion during coagulation in relation to the amount of prothrombin available for conversion to thrombin. Dicumarolized blood contains essentially normal amounts of L.F. (17). As is evident in Figure 3, dicumarol serum has a very strong restorative effect on aged plasma, more potent than normal serum (compare with data of Table II; also compare data in Table VII with those in Table V, all obtained on the same aged plasma). The same is true of dicumarol $BaSO_4$ serum compared with its normal counterpart. Again, as with normal serum, oxalate accelerated L.F. deterioration (Table VII).

TABLE VII

L.F. activity of dicumarol serum (D.S.) with and without oxalate

Aged plasma* plus:	Proth-T (*sec.*)
Saline	61
Fresh D.S.	11
Fresh oxal. D.S.	14
Fresh $BaSO_4$ D.S.	26
Fresh $BaSO_4$ D.S.-oxal.	39
D.S. kept 3 hrs., room temp.	13
Oxal. D.S., 3 hrs., room temp.	22
$BaSO_4$ D.S., 3 hrs., room temp.	36
$BaSO_4$ oxal. D.S., 3 hrs., room temp.	64

The prothrombic activity of the parent plasma was 11 per cent. Serum separated one hour after clotting. $BaSO_4$ S prepared with 100 mgm. $BaSO_4$ per ml.

* One volume of material to four of aged plasma (60 days at 4–5°C).

It was of interest to study the stability of L.F. in dicumarol serum as contrasted with normal serum. Accordingly, experiments were performed on the same sera recorded in Tables V and VII, after three days' storage at 4–5°C. Determinations were made with the same stored plasma which now had a prothrombin time of 80 seconds compared with 61 seconds three days earlier. The three-day-old normal serum lowered the prothrombin time from 80 to 64 seconds, whereas the three-day-old dicumarol serum reduced it to 32. Whether this difference, however, reflected a greater lability, or a lesser initial concentration, of L.F. in the normal serum is not clear.

The oxalated dicumarol serum became inert within three days.

Effect of thromboplastin on L.F. of dicumarol serum

As already shown, thromboplastin added to normal blood reduced serum L.F. activity. On dicumarol blood this effect was not observed. Serum derived from thromboplastin supplemented dicumarol blood contained the same L.F. activity as dicumarol serum derived without the thromboplastin. This was true whether non-oxalated was compared with oxalated serum, and $BaSO_4$ compared with non-$BaSO_4$ serum under the same conditions. It appears, therefore, that the effect of thromboplastin on the L.F. concentration of normal serum was related to the velocity and/or the amount of prothrombin conversion.

Again, in dicumarol-thromboplastin serum L.F. was much less stable in the presence of oxalate.

In these experiments 0.3 ml. of thromboplastin extract was added to 10 ml. of dicumarol blood containing 3 per cent prothrombin. The aged plasma on which the sera were tested had a prothrombin time of 37 seconds.

L.F. in hemophilic serum

Hemophilic serum has more restorative activity on aged plasma than normal serum (Table VIII), despite the fact that the interval between the shedding of the blood and the testing of the serum was

TABLE VIII

L.F. activity of hemophilic serum (S)

Aged plasma* plus:	Proth-T (*sec.*)
Exp't 1	
Saline	80
Norm. S	26
Hem. S	17
Norm. $BaSO_4$ S	34
Hem. $BaSO_4$ S	28
Exp't 2	
Saline	80
Hem. S	14
Hem. $BaSO_4$ S	28
Hem. Tpl.† S	38
Hem. Tpl. $BaSO_4$ S	55

* One volume of material added to four of aged plasma. Sera non-oxalated, separated one hour after clotting. $BaSO_4$ S obtained with 50 mgm. $BaSO_4$ per ml.

† One volume of thromboplastin extract to nine of blood.

[5] Obtained from patients with myocardial infarct treated with dicumarol for the prevention of thromboembolic disease.

inevitably greater as a result of the prolonged clotting time of the hemophilic blood. Also, $BaSO_4$ removes some restorative activity.

As in all the other experiments, oxalated serum from hemophilic blood had far less restorative ability than non-oxalated sera. This was true with whole as well as $BaSO_4$-adsorbed serum.

Supplementing hemophilic blood with thromboplastin, which accelerates coagulation, yields serum containing far less L.F. activity, practically the same as that observed in serum from normal blood similarly supplemented (Table VIII).

Serum L.F. in thrombocytopenia and thrombasthenia

These findings led to experiments in other pathological conditions where prothrombin conversion is reduced as a consequence of inadequate elaboration of thromboplastic material. It is evident (Table IX) that in thrombocytopenic purpura as well as in thrombasthenia, two disorders where prothrombin consumption is poor (15, 27), serum L.F. activity was abnormally elevated. In these cases, the $BaSO_4$ sera also were more potent in rectifying the retarded prothrombin conversion of aged plasma than $BaSO_4$ normal serum. In all instances, the $BaSO_4$ removed the large amounts of residual prothrombic activity characteristically present in serum in these disorders. Recovery in one patient with acute thrombocytopenia was associated with restoration of serum L.F. and prothrombin to normal values.

TABLE IX

Serum (S) L.F. in thrombocytopenic purpura and thrombasthenia

Pat. 1—W. Idiopathic thrombocytopenic purpura.
Pat. 2—E. Acute idiopathic thrombocytopenic purpura; quinidine sensitivity.
Pat. 3—G. Easy bruising, ecchymoses, normal to increased bleeding time, increased capillary fragility for years. High residual serum prothrombic activity. Platelet count: 122,000 per mm.³ blood (low normal).

Aged plasma* plus:	Prothrombin time (*sec.*) of mixture			
	Thrombocytopenia			Thrombasthenia
	Pat. 1	Pat. 2		Pat. 3
	36,000 plts.	*18,000 plts.*	*130,000 plts.*†	
Saline	66	67	68	76
Norm. Plas.	16	19	20	
Pat. Plas.	16	21	20	
Norm. S	31	15		21
Pat. S	16	13		18
Norm. $BaSO_4$ S	37	37	37	61
Pat. $BaSO_4$ S	23	26	33	50

* One volume of material to four volumes of aged plasma.
† After clinical recovery.

L.F. in "serum" from afibrinogenemic subject

Experiments performed on blood from a subject with afibrinogenemia [6] exclude the possibility that L.F. disappears during coagulation as a result of adsorption on the fibrin clot. Blood was drawn with and without added oxalate; the latter remained completely fluid indefinitely. One hour after the blood was shed, oxalate was added to the non-oxalated sample. The bloods were centrifuged. The plasma and "serum" were then incubated at 37°C for 15 minutes. Plasma prothrombin was normal by both one- and two-stage methods. The L.F. activity of the plasma also was normal. The "serum" contained no prothrombin by the two-stage procedure, and the L.F. activity of the $BaSO_4$ adsorbed "serum" was approximately 10 per cent of that of the parent plasma. Also of interest was the finding that the unadsorbed serum contained a normal amount of spca. These observations indicate that the disappearance of L.F. activity during coagulation is not related to formation of a fibrin clot. It may be concluded, furthermore, that spca elaboration is also independent of fibrinogen and its conversion to fibrin.

DISCUSSION

Although the importance of a non-prothrombin plasma constituent in the conversion of prothrombin by thromboplastin plus calcium is now definitely established, its role in this reaction is obscure. Quick and his collaborators, who have designated the entity by the term "Labile Factor" because of its lability, claim that it enters into the reaction stoichiometrically along with thromboplastin and calcium (28). On the other hand, the Wayne University group (Ware, Seegers, and colleagues), calling their substance "plasma Ac-globulin," consider it to be a relatively inert precursor of "serum Ac-globulin" to which it becomes converted by thrombin. Serum Ac-globulin then functions as an accelerator of the activation of

[6] We are indebted to Dr. Louis K. Diamond of the Children's Medical Center, Boston, for making blood, from this patient, available.

prothrombin (4, 29, 30). Owren, who holds similar views, terms his substance Factor V which is converted to Factor VI during coagulation, and which accelerates thrombin elaboration (12, 5).

Regardless of whether these moieties are identical (and it is becoming increasingly evident that they are), certain facts are clear. When L.F. is progressively reduced below 50 per cent of that in normal plasma, the prothrombin time increases (8). Prothrombin consumption is also curtailed (31). Thus, a given prothrombin time reflects the amount of L.F. (or plasma Ac-globulin and Factor V, if we assume that they are identical) as well as the concentration of prothrombin itself. Accordingly, in order to measure prothrombin as such, adequate quantities of this other factor must be assured. This point has received scant consideration in reports on serum prothrombin and on prothrombin consumption during coagulation, especially those based upon one-stage prothrombin determinations, where the velocity of prothrombin conversion is measured.

The foregoing data, in accordance with recent reports (28, 32), show that substantial amounts of L.F. disappear during normal coagulation. Depletion of L.F. begins before prothrombin falls off, but soon the rates with which both are consumed seem parallel. Accelerating and increasing prothrombin conversion with supplements of thromboplastin causes greater losses. Conversely, in hemophilia, thrombocytopenia, and thrombasthenia where prothrombin conversion is retarded or reduced, L.F. consumption appears reduced. Furthermore, curtailing the amount of available prothrombin also decreases the amount of L.F. consumed.

Accelerating coagulation and increasing prothrombin consumption in hemophilia by additions of thromboplastin induces greater L.F. consumption. It may, therefore, be concluded that this clotting factor is utilized in relation to the amount and/or velocity of prothrombin consumption. Furthermore, critically curtailing the concentration of L.F. limits the velocity as well as the amount of thrombin evolution (33). These findings lend support to the idea that L.F. enters into the reaction as a real participant rather than the alternative view that it is merely an "accelerator" acting outside the thrombin-forming system proper. This is in accordance with the interpretation of Quick and Stefanini (28) regarding L.F. and Lewis and Ferguson concerning Ac-globulin (34).

The amount of L.F. in serum is considerably influenced by the presence of decalcifying anticoagulants and the interval of time elapsing after coagulation. These facts must be considered in studies where these agents may be added to blood at various intervals after blood is shed in order to stop prothrombin conversion at various stages of the coagulation process. It is noteworthy that L.F. deteriorates more rapidly in oxalated than in citrated serum, as in plasma under the same condition.

All of these facts are pertinent to the determination of serum prothrombin and prothrombin consumption by the one-stage method. The orthodox procedure (18), modified by the addition of fibrinogen, will give grossly inaccurate results for two reasons: (*a*) During coagulation the L.F. disappears to a variable extent, which is considerably influenced by conditions of coagulation. Consumption of L.F. may reduce its concentration in serum to critically low levels, thus causing *elevated* prothrombin times. (*b*) Also, the prothrombin conversion accelerator (spca) which *evolves* during coagulation varies in its concentration in serum, again depending upon various conditions of coagulation (23). This clot-promoting factor increases the velocity of prothrombin conversion by thromboplastin and calcium, resulting in *shorter* prothrombin times than can be attributed to the prothrombin itself. It has already been shown that for this reason, one-stage determinations of the prothrombin in serum are inaccurate (15). Now there is additional basis for this conclusion.

Difficulties also arise in the determination of serum L.F. by the one-stage procedure. Clearly, the substance is measurable by its restorative activity on aged plasma in which this clotting factor has largely deteriorated. Thus, the restorative effect of serum might validly be considered a measure of L.F. if we could be sure that during coagulation alterations did not take place in components *other* than prothrombin or L.F., which might influence the velocity of prothrombin conversion in aged plasma. That such alterations do occur, however, has been shown conclusively. Spca evolves which, in the presence of L.F., will accelerate thrombin evolution in stored plasma (24). Seegers and his group had shown earlier that serum and serum

fractions activate and accelerate prothrombin conversion to a greater degree than plasma, and Owren has reported similar observations (12). Also, Milstone found that in the course of coagulation, "prothrombokinase" is converted to "thrombokinase" which is a potent accelerator of prothrombin conversion (35).

It is accordingly evident that some consideration must be given to clot-promoting agents which *arise* as a consequence of the coagulation process, and which may accelerate prothrombin conversion in aged plasma, simulating or enhancing L.F. activity. This is substantiated by the observations that $BaSO_4$ removes considerable restorative activity from serum despite the fact that this agent does not appreciably adsorb L.F. Since $BaSO_4$ removes spca (23) and thrombokinase (35), the effect on the restorative activity of serum is attributed to removal of this (these) substance(s). It should also be noted that $BaSO_4$ also adsorbs residual serum prothrombin. The restorative activity of $BaSO_4$ treated serum is, therefore, a more accurate index of serum L.F. than untreated serum.

The question arises whether serum Ac-globulin and Factor VI can be similarly excluded. According to Murphy and Seegers (36) and as has been confirmed by us (24), serum Ac-globulin is extremely unstable in normal human serum, virtually disappearing shortly after coagulation. The same is said to be true of Factor VI (12). This does not necessarily obviate the need for considering these factors because under abnormal conditions of coagulation they may be more stable, persisting in the serum. Indeed, substantial Ac-globulin activity is demonstrable in human serum prepared from blood allowed to clot slowly in siliconized tubes (36).

In a previous paper (24) evidence was presented which indicated that "serum" Ac-globulin probably comprises a mixture of plasma Ac-globulin (L.F.) plus spca, which together account for its greater prothrombin-converting activity than plasma Ac-globulin alone. The relatively low initial concentration of Ac-globulin in human plasma and its rapid depletion during coagulation explains the apparent extreme lability of "serum" Ac-globulin in human, in contrast to bovine, serum. On the basis of this interpretation, and assuming Factor VI to be identical with "serum" Ac-globulin, removal of spca by adsorption with $BaSO_4$ adequately takes care of clot-promoting activity of serum over and above that of its parent plasma as far as the restorative effect of serum on aged plasma is concerned.

In evaluating the restorative effect of serum as a measure of its L.F. content, consideration should also be given to the products of platelet disintegration which are said to accelerate prothrombin conversion in aged plasma (37, 38). It appears that platelets contain at least two clot-promoting substances, one which resembles Ac-globulin, and another which accelerates the interaction of thrombin with fibrinogen (39, 30).[7] Whether these appear, and persist, in serum as a consequence of clotting is not clear. Pending further investigation, it may be concluded that the restorative effect of $BaSO_4$ serum on aged plasma provides a suitable method for measuring serum L.F. By comparing the prothrombin times of mixtures containing various dilutions of $BaSO_4$ serum in aged plasma with similar mixtures containing the $BaSO_4$ parent plasma, the L.F. of the serum in per cent of that originally present in the blood can be determined.

CONCLUSIONS

1. Normal serum has as much, and frequently more, restorative activity than the parent plasma on the retarded prothrombin conversion of aged plasma.

2. This is largely due to spca which evolves during coagulation and which, together with whatever Labile Factor remains unconsumed, accelerates prothrombin conversion.

3. Removal of spca by adsorption with $BaSO_4$ yields a serum which still has some restorative activity. This activity is a measure of residual L.F. in serum, which under normal conditions is small.

4. L.F. begins to disappear from blood shortly after its withdrawal, even before detectable changes in the prothrombin concentration have occurred. Within 40 minutes consumption of both factors is virtually complete. Disappearance of L.F. during coagulation is unrelated to the conversion of fibrinogen to fibrin.

[7] It has previously been shown that spca does not act on this phase of the coagulation process (22, 24).

5. Accelerating coagulation and prothrombin conversion by thromboplastin supplements increases the amount of L.F. consumed.

6. Conversely, curtailing the amount of prothrombin converted to thrombin, by dicumarol or by pathological conditions such as hemophilia, thrombocytopenia or thrombasthenia results in sera containing abnormally large amounts of L.F. This could be corrected in hemophilia by thromboplastin supplements, but not in dicumarol blood.

7. These observations lend support to the concept that L.F. is an active participant in the prothrombin conversion reaction.

8. Serum L.F. seems more stable in citrate than in oxalate, but not as stable as in native serum. This is in accordance with its behavior in plasma under similar conditions.

9. These findings are discussed insofar as they relate to methods for the determination of serum prothrombin, serum L.F. and prothrombin consumption under normal and pathological conditions.

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ASSOCIATION ANNOUNCEMENT

The 43rd annual meeting of the American Society for Clinical Investigation will be held at the Chalfonte-Haddon Hall, Atlantic City, N. J., on Monday, April 30, 1951, at 9 a.m.

Other meetings to be held also at the Chalfonte-Haddon Hall are:

The 8th annual meeting of the American Psychosomatic Society, Satuday, April 28, 1951.

The National Meeting of the American Federation for Clinical Research, Tuesday, May 1, 1951.

The 64th meeting of the Association of American Physicians, Tuesday, May 1, and Wednesday, May 2, 1951.

ABSORPTION STUDIES USING PORTAL ANASTOMOTIC VEINS[1]

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In man the portal circulation, being without major and macroscopic connection with systemic veins, has been hidden from the clinical investigator. Even the intravascular catheter has not invaded the duplex circulations feeding the liver, but only its drainage system. Thus circulatory inaccessability of the key digestive gland in man has left it mysterious, a metabolic sphinx. Surgeons, deleting the spleen from persons with portal hypertension, have recorded sporadic data on portal pressure and blood gases (1, 2). Physiologists have estimated total hepatic blood flow in man by the bromsulphalein clearance test (3) which permits at least reasonable approximations. Sherlock and Walshe (4) added some facts by measuring portal blood from large superficial anastomotic veins in a single patient with cirrhosis. They reported observations in a patient with a large abdominal vein apparently draining blood from the upper segment of the alimentary canal into the right great saphenous vein. Their attention was concentrated on a comparison of systemic and anastomotic blood levels of glucose, galactose, fructose, fat and nitrogen at appropriate intervals after ingestion of measured quantities of these substances or their precursors, singly or in combination.

The ordinary collateral veins over the abdomen of cirrhotic patients have been studied in an effort to determine portal pressures (1, 5) and circulation time (6). Billings and DePree suggested that comparison of blood from the abdominal collaterals and systemic veins following administration of glucose orally might permit the differentiation of cirrhosis from other disorders giving rise to collateral vessels over the belly (7).

Myers (8) has made an effort at physiologic partition of hepatic arterial and portal venous components of the total blood flow to the liver by measuring the gas content of arterial, hepatic vein and caput medusae blood. If one assumes that there has been no reflux from hepatic artery into the portal anastomotic vessel and that the cases were not indeed instances of the Cruveilhier-Baumgarten syndrome (9), such observations may bring crucial evidence to impinge on Dock's surprising observations on postmortem infusion of hepatic artery and portal vein with kerosene (10).

We are reporting studies on two patients (see Appendix) with cirrhosis who had portal anastomotic veins large enough to permit multiple studies of absorption. One of these patients died and postmortem examination revealed an unusual connection between a branch of the superior mesenteric vein and the superficial abdominal vein which coursed upward and emptied into the superior vena cava via the left internal mammary and innominate veins. Details of the connections are given in Figures 9 and 10. Our observations were restricted in part by the chemical facilities at hand when the two patients were under observation almost simultaneously on the wards. By the time we had prepared to do more elaborate tests, one patient died in the hospital and the other went home and died. No others with such vessels have been found in our last 20 cirrhotic patients. Therefore, we are presenting our data as modest increments to knowledge which must be extended before we draw general conclusions.

METHODS

Our observations were made by comparing the levels of constituents of blood samples taken simultaneously from the antecubital and portal anastomotic vein at appropriate intervals after the ingestion of the mixture whose composition is listed in Table I. Studies included those on glucose (11), fructose (12) and vitamin A (13,

[1] Aided in part by the Medical Trust Fund of the Department of Internal Medicine, and research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service, and the Commission on Liver Disease of the Armed Forces Epidemiological Board.

TABLE I

Contents of test meal

60 grams of protein (skim milk and skim milk powder)
80 grams of fat (cream)
50 grams of glucose
50 grams of fructose
500 mg. of ascorbic acid
5 mg. of thiamine
10,000 units vitamin A (M. W.)
25,000 units vitamin A (N. N.)
250 ml. water

14), neutral fat (15), thiamine (16, 17), ascorbic acid (18), amino acid nitrogen (19) and urea nitrogen by the urease method. In no case was the error of the method more than ± 5%. We did all tests in duplicate except for glucose and urea nitrogen. The amount of blood which could be withdrawn from the abdominal vein sometimes limited the number of tests it was possible to do. Some samples were used for tests which would have been omitted had we been able to predict results. We collected further data on oxygen saturation before and after eating; on circulation time and venous pressure. We did the absorption studies the day following paracentesis in order to reduce by some degree the mechanical interference with alimentary function and to make the abdominal vein more accessible. For a period of one week prior to each absorption study, both patients were fed a high-carbohydrate, high-protein and unrestricted-fat diet. Supplemental vitamins were furnished in the form of multi-vitamin capsules and crude brewer's yeast. In addition, subject M. W. received thiamine, riboflavin and vitamin K.

OBSERVATIONS

The information collected in this study is presented in a series of charts.

Carbohydrate

Blood samples were taken after a 14-hour fast, and 50 grams of glucose were given orally at zero time. From Figure 1 the following facts are noted.

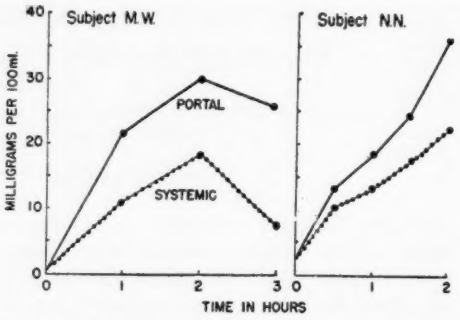

FIG. 2. COMPARISON OF PORTAL AND SYSTEMIC BLOOD FRUCTOSE LEVELS FOLLOWING INGESTION OF TEST MEAL

(*1*) The fasting level of glucose in abdominal and antecubital vein was the same. (*2*) In all tests the level of glucose rose more rapidly, and for 1½ to two hours was sustained consistently at higher levels in the abdominal vein than in the vein of the arm. In the first test in subject M. W. the level of glucose in the abdominal vein and arm vein was elevated above the normal at the two-hour period. In the second test the abdominal vein blood showed higher glucose values at the end of two hours with the levels both approaching normal at the end of three hours. The third test three months later when the patient was clinically much worse showed a high glucose level in the abdominal vein blood at the end of two hours. No later determinations were made. (*3*) In subject N. N. the arm and abdominal blood glucose levels were slightly elevated with the abdominal blood level being higher at the end of two hours. There was no regularity of time relations for peak of glucose

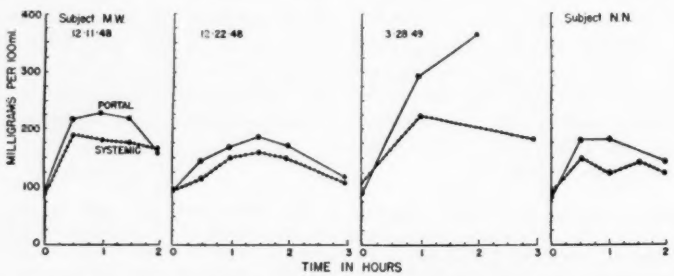

FIG. 1. COMPARISON OF PORTAL AND SYSTEMIC BLOOD GLUCOSE LEVELS FOLLOWING INGESTION OF TEST MEAL

in the abdominal vein, although the average time for peak elevation was somewhat later (75 min.) than for the arm vein (53 min.). The peaks in all four tests differed varying from one-half to two hours. The fasting blood glucose levels were normal in both subjects. (*4*) Fructose (Figure 2)—Both subjects had normal fasting levels and a decreased tolerance. The absolute value of the blood fructose was less than that of glucose although proportionately the rise of fructose was greater than that of glucose. Except for the fasting samples, the fructose levels in the abdominal vein were higher at all times in both subjects. Peak level in abdominal and arm vein blood occurred at the end of two hours. No later determinations were carried out on subject N. N.

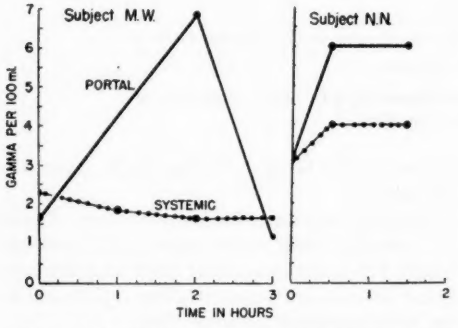

FIG. 3. COMPARISON OF PORTAL AND SYSTEMIC BLOOD THIAMINE LEVELS FOLLOWING INGESTION OF TEST MEAL

Water Soluble Vitamins

Five mg. of thiamine were given orally as the test dose. There was no clinical clue to specific thiamine deficit but the dietary history suggested that there was moderate thiamine undernutrition.

From Figure 3 it may be seen that: (*1*) Anastomotic vein blood rose to much higher levels than did blood from the arm vein. (*2*) Rise in systemic vein was absent or very slight. (*3*) The curves do not have enough points to describe a general pattern of behavior. (*4*) In one subject the thiamine level of anastomotic vein blood had returned to the control level at three hours.

Ascorbic acid was given orally in the amount of 500 mg. Figure 4 reveals that: (*1*) There was no substantial difference in the levels from portal and systemic blood in fasting or postprandial samples. (*2*) Portal blood was regularly higher than systemic blood, though not to a significant degree. (*3*) No fall in level had occurred at the end of two or three hours in the two cases.

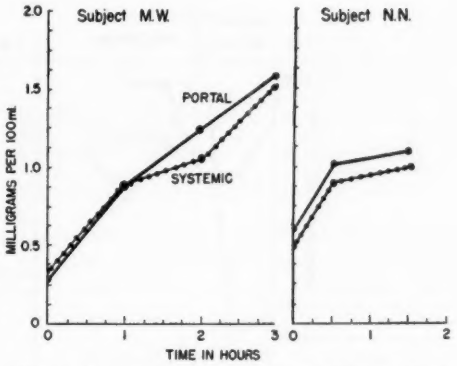

FIG. 4. COMPARISON OF PORTAL AND SYSTEMIC BLOOD ASCORBIC ACID LEVELS FOLLOWING INGESTION OF TEST MEAL

Vitamin A

Vitamin A was given orally, 10,000 units, to subject M. W. and 25,000 units to subject N. N. Figure 5 reveals: (*1*) Similar fasting levels in portal and systemic blood. (*2*) More rapid and higher rises in portal than in systemic blood. (*3*) Return to control values at three hours in one subject.

Neutral Fat

Neutral fat was given as 80 grams of cream. Figure 6 indicates: (*1*) A distinctly higher systemic than portal fasting level. (*2*) Irregular

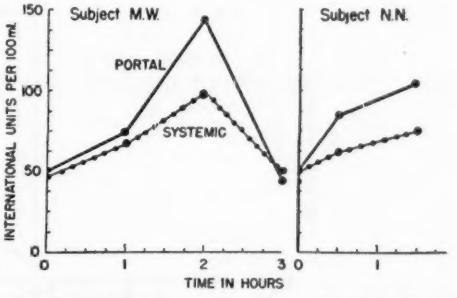

FIG. 5. COMPARISON OF PORTAL AND SYSTEMIC BLOOD VITAMIN A LEVELS FOLLOWING INGESTION OF TEST MEAL

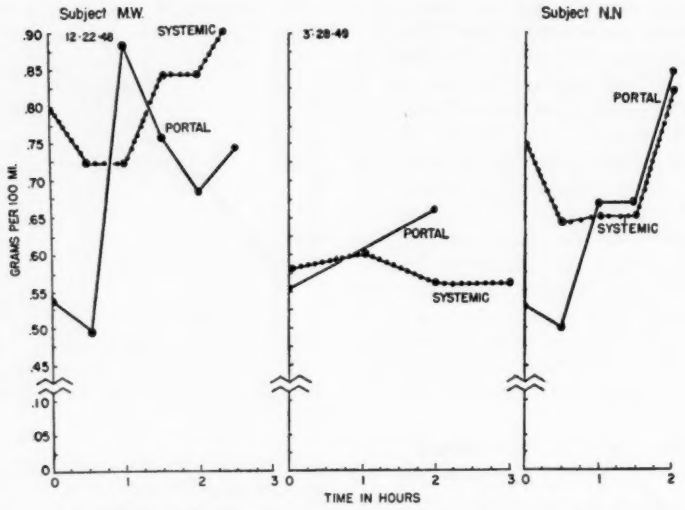

FIG. 6. COMPARISON OF PORTAL AND SYSTEMIC BLOOD PLASMA LIPID LEVELS FOLLOWING INGESTION OF TEST MEAL

rises in which portal blood levels rose at a more rapid rate. (*3*) Two of the three tests indicate a delayed rise of lipid in systemic blood.

Nitrogen

Sixty grams of protein in the form of skim milk and skim milk powder were given. Figure 7 for amino acid nitrogen indicates that: (*1*) The tests showed no clear difference in portal and anastomotic vein levels of amino acid nitrogen though there was a tendency for a peak to occur at two to 2½ hours. (*2*) The levels varied erratically.

Figure 8, indicating blood urea nitrogen, shows: (*1*) Similar fasting levels in portal and systemic blood; (*2*) a rise in systemic blood urea nitrogen at one hour, in one instance falling rapidly and in the other sustained for some time.

Circulation Time

Circulation time was determined by the decholin method. The results are in Table II. The

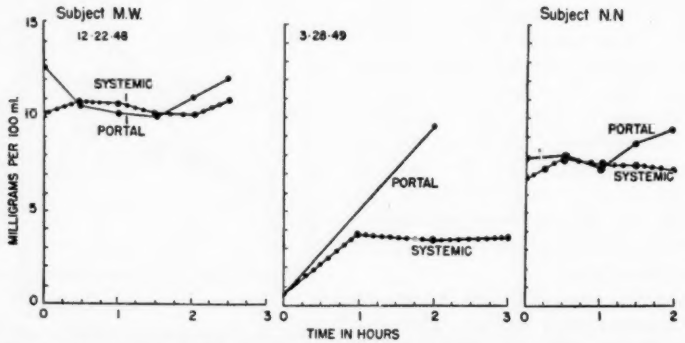

FIG. 7. COMPARISON OF PORTAL AND SYSTEMIC BLOOD AMINO ACID LEVELS FOLLOWING INGESTION OF TEST MEAL

blood from the abdominal vein reached the tongue in 11 and 14 seconds, distinctly less than the arm to tongue time. Femoral vein to tongue time was at the upper range of normal.

Venous Pressure

The determinations of portal vein pressure are compared with those from the antecubital and femoral vein in Table III. The right atrium was the standard zero reference.

In one subject (N. N.) studies of blood gases were attempted in the fasting state and during absorption. The necessity of producing stasis in order to obtain blood from the portal vein, which

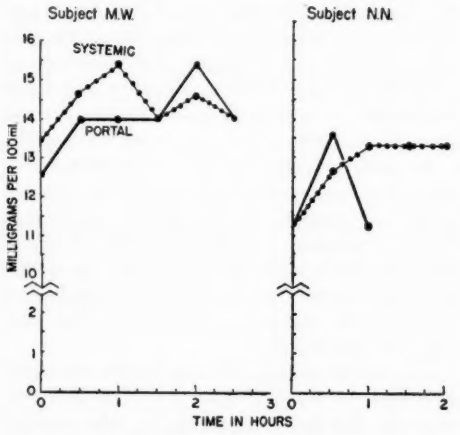

FIG. 8. COMPARISON OF PORTAL AND SYSTEMIC BLOOD UREA NITROGEN LEVELS FOLLOWING INGESTION OF TEST MEAL

was copied as far as possible in taking blood from the arm, makes the data presented in Table IV of questionable accuracy. The blood from the portal anastomotic vein was regularly of a brighter red color than that from the arm vein.

DISCUSSION

Although studies of absorption were made by analysis of portal vein blood in animals (20) in 1877 no similar efforts to evaluate the phenomena of absorption in man have been made until recently. While the results of the biochemical tests on various substances are roughly comparable in the two subjects, and postmortem observations

TABLE II

Circulation time in seconds (decholin)

	M. W.	N. N.
Anastomotic vein to tongue	11*	14
Arm to tongue	16	17
Femoral vein to tongue	26	27

* Each number is the average of two determinations.

in one of them established the anatomical relations of the portal anastomotic vein, the data are scarcely adequate for broad generalization. Together with the patient reported by Sherlock and Walshe (4) whose anastomotic vein carried blood down to the saphenous vein, three subjects with large portal anastomotic veins have been investi-

TABLE III

Venous pressures (cm. water) referred to right atrium as zero point

	M. W.	N. N.
Arm	11*	9
Arm (after R.U.Q. pressure)	12.5	9
Femoral	11.5	9
Femoral (after R.U.Q. pressure)	19	13
Anastomotic vein	22	19
Anastomotic vein (after R.U.Q. pressure)	23	19
Anastomotic vein (after Valsalva procedure)	26	25

* Each number is the average of two determinations.

gated. Our patients both had a similar pattern of superficial venous drainage in a cephalic direction by a large anastomotic vein. This was demonstrated by local pressure which indicated that the vein filled more rapidly from below than from above; and the exaggerated prominence produced by the Valsalva procedure. Because we

TABLE IV

Oxygen saturation (subject N. N.)

	Volumes %	Saturation (%)
Fasting Blood		
Arm vein	7.0	38.8
Anastomotic vein	14.0	82.5
After Meal		
Arm vein	5.0	27.8
Anastomotic vein	14.2	79.0
Oxygen capacity	18.0	

could not estimate the moiety of portal blood carried in this vein, it is idle to speculate upon the quantitative aspects of the data. They merely indicate trends. Another limitation in the study is the reliability of the venous blood from the arm, representing not mixed venous blood but a particular sample. In essence we are comparing the constituents of two different effluent streams of blood, but we cannot relate the observed data to total quantities. The effect of the water of the test feeding added another variable.

A number of factors militate against the application of our findings to the normal processes of absorption in man. The metabolic function of the cirrhotic liver is impaired, but no single test or clinical finding permits precise quantitation of the several disturbances which would influence absorption and hepatic metabolism. The higher pressure of blood in the portal circuit well known clinically, demonstrated at operation (1, 21) and measured directly in our subjects might be expected to diminish or retard absorption from the gut. It is not known to what extent commingling of arterial blood from the hepatic artery with venous blood from the intestine occurs, or even that it has any quantitative significance. Our observations, therefore, though they provoke speculation, provide no conclusive solution to the problems they bear upon. Because of the anatomical relations in subject M. W., it is probable that significant admixture with systemic blood did not occur.

Carbohydrate. It is the consensus of investigators that glucose is absorbed by a phosphorylation mechanism with hexose phosphoric acid serving to transport glucose across the mucosa where glucose again is formed and transported as such to the liver. Our studies (Figure 1) suggest that this process is protracted over periods of at least two hours. In all observations the curves of the systemic glucose and fructose blood levels are typical of those found in liver disease, namely, normal fasting levels with decreased tolerance after ingestion of the sugar. The elevated levels are attributed to impairment of the liver's function in carbohydrate regulation. The prolonged elevation likewise may result from hepatic malfunction and probably impaired intestinal absorption as well. The earlier and higher rise and the more rapid return to normal of glucose in contrast to fructose is in keeping with the fact that gastro-intestinal absorption of glucose is more rapid than that of fructose although the rates of utilization in the liver are similar (*22*). The factors of tissue utilization of glucose, possible glucogenesis from the protein in the test feeding, and the effect of fat in delaying absorption could not be appraised.

Water soluble vitamins. Thiamine levels in portal blood rose rapidly whereas the rise in systemic blood was meagre or did not follow (Figure 3). Perhaps this merely reflects thiamine depletion in the tissues. We have no knowledge of what metabolic alterations were produced because of the bypass of so much portal blood to the general systemic circulation before passage through the liver. The fact that portal blood levels rose without a similar rise of systemic blood thiamine raises a question about the usual interpretation of such flat tolerance curves as indicative of reduced absorption; and it must be concluded that thiamine was utilized, stored, destroyed, or excreted rapidly.

Ascorbic acid levels followed a different course; and there was no substantial difference between portal and systemic blood samples. One could interpret this as an indication of a state of saturation, though the initial levels are below the usually accepted normal range. Another assumption might be that the use or elimination of ascorbic acid was diminished.

Fat soluble vitamins. To some extent the curves of vitamin A levels (Figure 5) were similar to those for glucose. Subject N. N., who received 25,000 units, had less of a rise than subject M. W., who received 10,000 units though the time intervals of sampling and the duration of the tests differed.

Lipids. There was a sharp disparity between portal and systemic blood levels for neutral fat in the fasting state, suggesting that the liver was liberating fat into the blood stream, or possibly that it was removed somewhere in the long circuit between hepatic vein and the portal collecting system. In two instances there was an initial fall and then a sharp rise in portal blood to levels somewhat higher than the simultaneous systemic blood level.

Nitrogen. Figures 7 and 8 exhibit data from studies on portal and systemic blood urea nitrogen and amino acid nitrogen. Similar levels for amino acid nitrogen occurred during the early stages of

absorption and not until the second hour was the level in portal blood significantly higher than in systemic vein blood at a time when the breakdown of protein to amino acids in the gut was well advanced. The curves for urea nitrogen (Figure 8) suggest several interpretations. In M. W. it may be supposed that the formation of urea from amino acid was advancing rapidly and reached an early peak, after which the process declined or excretion increased. In N. N. the curves may be taken to suggest the absorption of urea, or its formation in the gut, though the differences are not striking, and the variations suggest that the results may be in error.

Oxygen. It is probable that technical difficulties with the collection of blood from the anastomotic vein, the necessity for using pressure and stasis and efforts to duplicate the situation in the vein of the arm make the quantities unreliable. Nevertheless, in each sample taken from the abdominal vein it was obvious that the blood had the bright red color nearly that of arterial blood. By ordinary inspection, and by the use of red goggles, the anastomotic vein appeared less dark than neighboring veins coursing up over the abdomen. It failed to show up as well as we expected with infrared photography. We have demonstrated in some other patients with cirrhosis identical blood glucose levels in arm and abdominal collateral veins after a test meal, and in spite of the report of Billings and DePree (*7*), we are of the opinion that some of the prominent abdominal veins in cirrhotics carry unmixed systemic blood from the lower extremity. The high oxygen content of the anastomotic vein may indicate (*1*) a reflux of hepatic artery blood from the liver, (*2*) an open state of the numerous arteriovenous connections in the gut, or (*3*) very modest oxygen consumption by the gut during absorption. Whether or not these speculations are correct or some other cause exists, the finding casts a reasonable doubt on estimates of the moiety of blood supplied to the liver by hepatic artery and portal vein where gauged by gases from portal vein blood (*8*). The very low levels of oxygen in systemic venous blood are puzzling although reduced oxygen content of the blood has been reported in cirrhotics (*23*).

Venous pressure. The dynamic state of the portal circulation, a low pressure system suspended between two capillary beds and thus indeed a veritable *rete mirabile*, has been well hidden. Our only information in man has come from surgeons who have measured pressure directly at laparotomy, usually in subjects with portal hypertension. Observations on abdominal veins by Levy and Burch (*5*) give no indication that portal anastomotic veins were used and the technique of occlusion used by Davidson, Gibbons and Faloon (*24*), while indicating the pressure which may be reached, introduces an artifact if any appreciable quantity of blood flow is obstructed. Our observations were carried out the day after abdominal paracentesis, when there was relatively little distention, even though some ascitic fluid remained. The portal pressure of 22 and 19 cm. of water in the two subjects was significantly higher than the systemic venous pressure (Table III). The Valsalva maneuver and hepatic jugular reflux test produced the expected changes.

Circulation time studies (Table II) suggest that blood from the anastomotic vein traversed a shorter distance or moved more rapidly than that from the arm.

RESUMÉ

The data on absorption and portal circulation gained from two subjects with portal anastomotic veins who were fed a mixture of test substances permit the following tentative statements:

1. Carbohydrate was absorbed rapidly from the upper part of the alimentary canal and the high level was sustained 90 minutes or more.

2. Thiamine, though it reached high levels in portal blood, did not rise significantly in systemic venous blood.

3. Ascorbic acid levels did not differ in portal and systemic blood during the rise following absorption.

4. Vitamin A was absorbed rapidly from the upper alimentary canal and systemic blood levels rose more slowly and not to the same degree.

5. The fasting level of lipids initially was lower in portal blood than in blood from the arm vein. In two instances there was an early fall followed by a sharp rise.

6. Amino acid rise in portal vein was delayed, variable and of small degree. Urea nitrogen was higher in systemic than portal blood during much of the absorption period, but the erratic behavior precludes analysis.

TABLE V

Subject	M. W.	M. W.	M. W.	N. N.
	12–11–48	*12–22–48*	*3–28–49*	*6–28–49*
R.B.C.	4.2	4.0	4.9	4.0
Hg. (*gm. per 100 ml.*)	13	13	14	11.9
W.B.C.	7,000	6,300	9,100	11,690
Total protein (*gm. per 100 ml.*)	5.4	6.5	6.2	6.4
Albumin (*gm. per 100 ml.*)	2.5	3.0	2.9	3.2
Globulin (*gm. per 100 ml.*)	2.9	3.5	3.3	3.1
Urine bilirubin	0	0	0–3+	4+
Urine urobilinogen	4+	4+	4+	1+
Van den Bergh (*1 min. mg. per 100 ml.*)	—	0.5	—	—
Van den Bergh (*30 min. mg. per 100 ml.*)	3.3	2.7	1.8	6.4
Alk. phosphatase (*Bod. units*)	5.2	6.4	8.4	17.2
Ceph. flocculation	4+	3+	2+	1+
Thymol turbidity units	*22*	*26*	*27*	*2.6*
Zinc sulfate turbidity units	—	25	27	11.0
Cholesterol (*mg. per 100 ml.*)	187	233	324	168
Cholesterol esters (*mg. per 100 ml.*)	83	152	219	75
Prothrombin time (*sec.*)	*46*	—	*44*	*30*
Prothrombin time (*sec.*) (control)	36.3	—	41.7	29
Bromsulphalein retention (*5 mg. per kilo.—45 minutes*)	44%	—	29%	42%
17-Ketosteroids (*mg. per 24 hr.*)	—	—	2.2	1.4

7. Oxygen content of portal vein blood was elevated, portal venous pressure was higher than systemic pressure and circulation time from anastomotic vein to tongue shorter than from arm to tongue.

8. These observations leave unanswered more questions than they illuminate. They emphasize a clinical finding first used by Sherlock and Walshe to solve some of the metabolic problems concealed in the hitherto inaccessible reaches of the portal blood system in man.

APPENDIX

Case Reports

M. W., a 43 year old railroad station agent, had three admissions to the Medical Service of the University Hospitals: 8 November—30 December, 1938; 21–30 March, 1949; and 30 September—5 October, 1949. His story on the first admission included a bout of "yellow jaundice" in 1932 and no recent but probably previous excessive use of alcohol. His illness began insidiously in March 1948, with discomfort in both flanks, indigestion, sporadic nausea and vomiting. Within two months his belly became swollen, his appetite declined and food gave rise to a sensation of bloating. Though there was weakness, malaise and easy fatigability, his weight rose from 155 to 162 pounds. Hemorrhoids appeared during the month before admission. He had become aware of the advent of vascular spiders within six weeks of the onset of his illness. During the month before admission his ankles became swollen, especially in the evening. Prior to his illness his diet was adequate. Nothing else of consequence was found in his past history or in the family history.

Examination disclosed a well-built, undernourished man without jaundice. He was alert, cooperative and in no acute distress. His skin had a slatey gray cast and was liberally sprinkled with vascular spiders though there was no palmar erythema or gynecomastia. Pectoral,

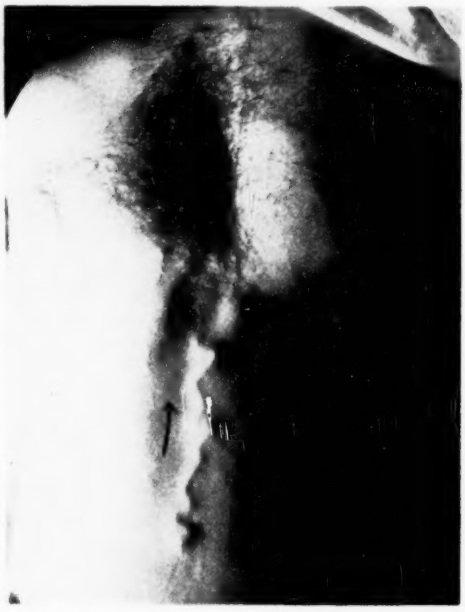

FIG. 9a. PHOTOGRAPH OF ANTERIOR ABDOMINAL WALL VEIN IN PATIENT M. W. DURING A VALSALVA PROCEDURE
Arrow marks direction of blood flow.

pubic and axillary hair was ample. A few moist rales were heard at the right base posteriorly where impairment of the percussion note was found. The diaphragms were elevated and the abdomen was much distended. A fluid wave was found. There were a number of dilated veins coursing upward over the abdomen; and a very large one was found emerging in the midline to follow a tortuous course upward to the region of the xiphoid where it disappeared below the rib margin to the right. The Valsalva procedure caused it to stand out elegantly in relief (see Figure 9a). After paracentesis of 6 liters the spleen was felt, but not the liver. There were external hemorrhoids. Other features of the examination were not unusual.

Course: During the first admission he was kept in bed and given a diet high in protein and carbohydrate with supplemental vitamins. Salt was restricted and mercurial diuretics were used repeatedly. Five paracenteses, each yielding 5 to 6 liters, were done between 11 November and 28 December.

There was gradual though not very substantial improvement and he was sent home on the same regimen where he required abdominal paracentesis at about ten-day intervals.

Second admission: The physical signs had not changed appreciably. Laboratory data are included in Table V. Two paracenteses were done yielding 2 liters each and absorption studies were carried out. He was discharged on the same regimen as before.

Interval: During his stay at home he continued to become weaker but adhered to his diet and medication, needing paracentesis at weekly intervals. A few days before his final admission he suddenly became stuporous. Bright red blood was passed by rectum several times.

Final admission: It was suspected that some of the lethargy might have resulted from barbiturates which he had been given just prior to hospitalization. He became alert enough to eat after 4 liters of slightly turbid ascitic fluid were removed, but soon lapsed back into stupor. He was then sustained by intravenous glucose and

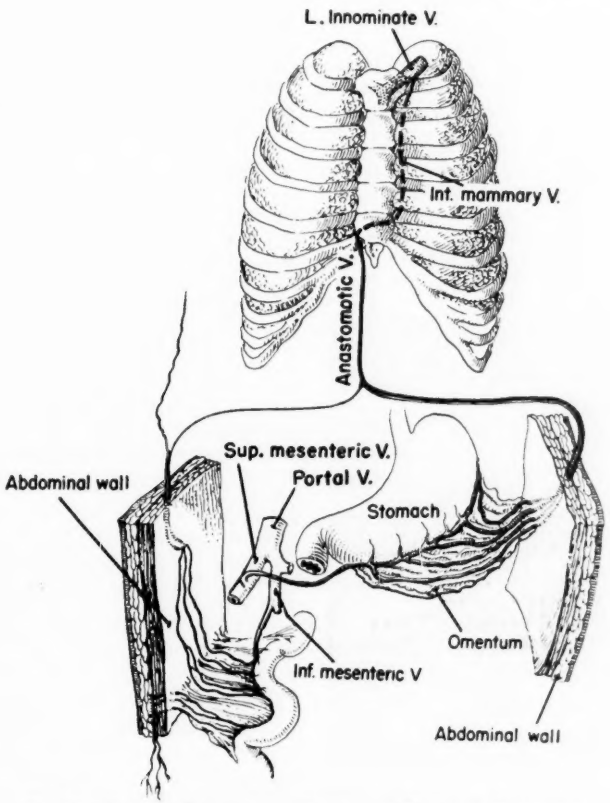

FIG. 9b. DIAGRAM ILLUSTRATING ORIGIN AND COURSE OF ABDOMINAL ANASTOMOTIC VEIN IN PATIENT M. W.

salt-free albumin. Clinical jaundice became apparent and bile appeared in the urine. He had vomited bloody fluid on two occasions shortly before he died.

Laboratory studies are summarized in Table V.

Autopsy: The significant findings were severe portal cirrhosis with ruptured esophageal varices and hemorrhage into the gastro-intestinal tract. A chronic congestive splenomegaly and abdominal ascites of approximately 1500 ml. of bile-stained fluid were present. There was great dilatation of the esophageal, gastric, paraumbilical and hemorrhoidal veins. The parietal peritoneal veins were distinctly dilated, particularly of both lateral peritoneal gutters and in the left upper abdomen where the greater omentum was adherent. The dilated omental veins originating from the stomach communicated with the lateral parietal veins in the left upper abdomen (Figures 9a, b and 10a, b). Most of this latter plexus of veins formed a single vein, which ran ventrally and emerged superficially about 2 inches above the umbilicus. There it received a small tributary from the dilated plexus of veins in the right peritoneal gutter which in turn communicated with intestinal veins via the mesentary. After becoming superficial the abdominal vein coursed cephalad and pierced the deep tissues in the region of the xiphisternum slightly to the right. It then turned to the left and joined the left internal mammary vein on the posterior surface of the anterior chest wall. This in turn entered the left innominate vein (Figures 9 and 10).

FIG. 10a. PHOTOGRAPH SHOWING ANASTOMOTIC VEIN PIERCING ANTERIOR SURFACE OF CHEST WALL IN REGION OF XIPHISTERNUM

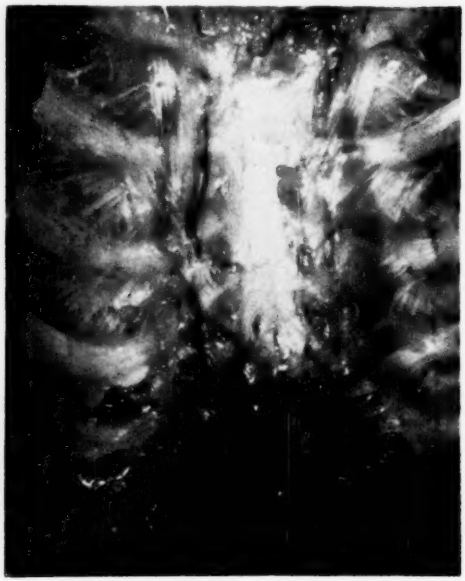

FIG. 10b. PHOTOGRAPH OF POSTERIOR SURFACE OF ANTERIOR CHEST WALL SHOWING LEFT INTERNAL MAMMARY VEIN WHICH HAS JUST BEEN JOINED BY THE ANASTOMOTIC VEIN DESCRIBED IN FIGURE 10a.

N. N., a 51 year old German truck driver, was admitted to the Medical Service of the University Hospitals 27 June and discharged 23 August, 1949. There had been a brief visit to the Medical Out-Patient Clinic in October, 1938, when an anxiety state was suspected. At that time he was drinking beer occasionally. There was no evidence of liver disease. He reported a severe bout of malaria with coma two years previously.

During the year before admission insidiously beginning fatigue had progressed to a state of incapacity for the last three months. One month before entry abdominal discomfort, bloating and anorexia dominated the scene. There was diarrhea. Chilly feelings and fever occurred repeatedly until a week before admission when he took quinine. The urine became dark three weeks before admission but he had not noticed jaundice or itching. His weight declined from 170 to 135 pounds in spite of increase in his girth which interfered with comfortable breathing.

Examination revealed an ill man, whose swollen belly contributed to orthopnea. The skin was not jaundiced but exposed areas were tanned. Vascular spiders were scattered over the chest, arms and shoulders. The sclerae were icteric. Retinal vessels showed moderate sclerosis. The left side of the chest was restricted in movement and the trachea deviated to the right. Signs of fluid were noted on the left. A harsh systolic murmur, transmitted into the neck, was heard in the second and third interspaces on the right; and P_2 was accentuated. Fluid distended the abdomen. The firm irregular edge of the liver was felt 5 cm. below the rib margin on the right. The spleen was not felt. Pitting edema of the ankles was noted. Body and axillary hair were plentiful. A large anastomotic vein arising midway between umbilicus and

xiphoid carried blood upwards. Large hemorrhoids were found.

Laboratory studies are summarized in Table V.

Treatment consisted of bedrest, high-protein, high-carbohydrate and low-salt diet supplemented with vitamins. Crude liver extract and mercuhydrin were given at frequent intervals. The left side of the chest was tapped on two occasions and 800 ml. of fluid removed each time. A number of abdominal paracenteses were performed with removal of 1–2 liters. Absorption and pressure studies were done on 15 August, 1949. The jaundice diminished and there was some clinical improvement, but he was still accumulating fluid at the time of discharge. He died at home on 1 September, 1949.

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EFFECT OF CORTISONE ON ACUTE STREPTOCOCCAL INFECTIONS AND POST-STREPTOCOCCAL COMPLICATIONS [1]

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It has been established that adrenal hormones play an important role in the defense reaction of the body to stress (1). A common stress stimulus in man is that produced by infection with group A streptococci. Streptococcal infections assume especial importance because they precede acute rheumatic fever, a disease which responds favorably to treatment with the adrenal hormone, cortisone (2–4). An epidemic of streptococcal exudative tonsillitis and pharyngitis afforded the opportunity to obtain data on the effect of cortisone therapy not only on an acute bacterial infection and the immunological response of the host, but also on the subsequent occurrence of acute rheumatic fever.

DESCRIPTION OF STUDY

This study was conducted at Francis E. Warren Air Force Base, Wyoming, from February 20 to April 17, 1950. All patients entering the hospital with respiratory infections were examined within a few hours after admission. Since it was desirable to include in the study only those patients in the early phase of a streptococcal respiratory infection who would be available for follow-up examinations, the following criteria were employed: (*a*) the presence of exudate on the pharynx or tonsils, (*b*) a total leucocyte of 10,000 or greater, (*c*) an illness which began less than 31 hours before admission to the study, and (*d*) availability for follow-up studies for at least four weeks.

One hundred and seventy-four patients fulfilled the above criteria and were divided into treated and control groups on the basis of their Air Force serial number. Eighty-seven patients had numbers ending in the digits 5–9 and received treatment; 87 with numbers ending in the digits 0–4 received no specific therapy and composed the control group.

Cortisone acetate was injected intramuscularly into the gluteal region according to one of the two treatment schedules. The first 17 patients received a total of 500 mgms in 50 mgm doses. The first injection was given at the time of admission to the study and subsequent doses were administered at 12 hour intervals. The remaining 70 cases were given a total of 600 mgms in 100 mgm doses. The initial injection was given at the time of admission to the study, the second and third at 9 a.m. and 9 p.m. on the second hospital day, and the remaining three at 9 p.m. of the third, fourth and fifth days. Control patients received 0.85 per cent sodium chloride injections by the same schedules. During hospitalization no antipyretics or other medications were given except codeine for relief of severe headache.

Patients were examined daily through the sixth day of illness by one member of the professional staff who had no knowledge of the treatment being administered. Symptoms were recorded for each 12 hour period during the first two days of illness and daily thereafter. Physical signs were recorded daily. Oral temperatures were taken every four hours. Total leucocyte counts and cultures of the tonsils and pharynx were obtained each day. Sera were obtained on the first and sixth days of illness. An electrocardiogram was recorded soon after the acute symptoms had subsided.

Subsequent examinations were conducted during the third, fourth and fifth weeks following the onset of the streptococcal illness. The patients were questioned concerning post-streptococcal complications. Those with symptoms were examined and admitted to the hospital for further study. An electrocardiogram was taken approximately 21 days after onset. In addition, at each of the weekly examinations, serum and a culture of the throat were obtained.

All cultures were examined for *beta*-hemolytic streptococci which, when present, were isolated in pure culture. The first and last strains isolated during hospitalization and all strains obtained at each subsequent examination were grouped and typed according to the methods of Maxted (5, 6) and Swift, Wilson, and Lancefield (7). The acute and convalescent antistreptolysin "O" titers of all sera from each patient were determined concomitantly by a modification of the technique of Hodge and Swift (8).

RESULTS

That the patients in this study had streptococcal disease is indicated by the demonstration of a sig-

[1] This investigation was supported through the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, Office of The Surgeon General, Washington, D. C.

[2] Capt. MC, AUS.

nificant rise [3] in the antistreptolysin titer in the sera of 86 per cent of the control group. In addition, group A streptococci were isolated from the initial cultures of the tonsils and pharynx of 89 per cent of all patients and the symptoms and physical signs were characteristic of streptococcal infections (9). The data presented in Table I show that the two groups of patients were essentially comparable.

The effect of cortisone on selected symptoms and physical signs is presented in Figures 1 and 2.[4] Little or no effect on the acute illness was demonstrated. The symptom, sore throat, persisted somewhat longer in the control group, being present on the sixth day of observation in 20 per cent of the control patients and 6 per cent of the treated cases. Chilliness, headache and malaise occurred with equal frequency in the two groups. No difference was noted between the treated and control patients in the time of disappearance of physical signs.

TABLE I

Comparability of the 87 cases in each of the treated and control groups

	Cortisone group *per cent*	Control group *per cent*
History of tonsillectomy	26.4	31.0
Age 17–21	77.0	74.7
*Symptoms:**		
Chilliness	65.5	75.8
Feverishness	82.7	86.2
Malaise	41.3	56.3
Sore Throat	82.7	85.0
Headache	86.2	80.4
Physical signs:†		
Enlarged or tender cervical nodes	91.8	90.8
Lymphoid hyperplasia	37.9	37.9
Laboratory:		
Group A streptococci on admission	89.7	88.5
Type 14	54.0	65.5
Type 24	17.2	10.3
Type 6	9.1	9.1
Leucocyte count 12,000 or greater	91.9	86.2
Antistreptolysin titer—125 units or less on admission	68.9	67.8

* Symptoms occurring during first 12 hours of illness.
† Physical signs present on admission examination.

The number of patients with fever is recorded in Figure 3. At each four hour time period prior to treatment, the incidence of fever was the same in the two groups. Following therapy, the per

[3] A significant rise is considered to be two dilution increments when the convalescent titer is compared to the acute titer.

[4] Observations made after the development of purulent complications in four patients from the control group were not included in this analysis.

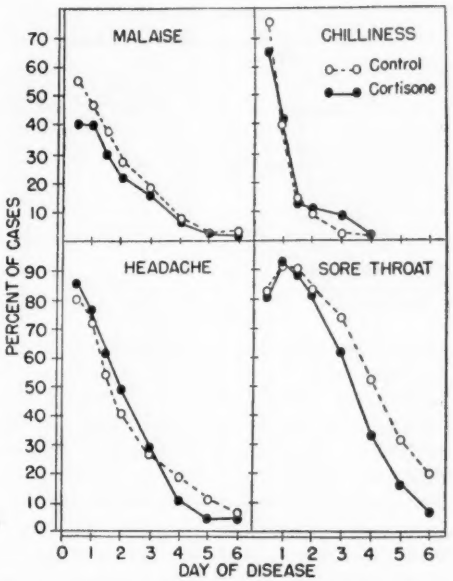

FIG. 1. EFFECT OF CORTISONE THERAPY ON THE SYMPTOMS OF STREPTOCOCCAL TONSILLITIS AND PHARYNGITIS

cent of patients with fever was greater in those who received cortisone than in the control group. This difference, evident up to the third day of treatment, was as great as 26 per cent.

There were 15 patients who developed a complication during the period of treatment with cortisone or saline injections (Table II). Two of the

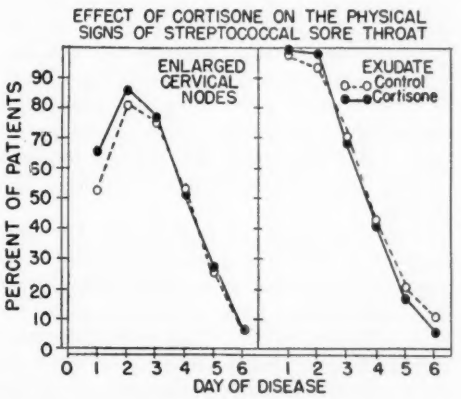

FIG. 2

TABLE II

The effect of cortisone on the complications of streptococcal sore throat

Complication	Cortisone group (87 cases)	Control group (87 cases)
Suppurative:		
A. During treatment		
1. Peritonsillar cellulitis	0	3
2. Otitis media	4	7
3. Appendicitis	1	0
B. Following treatment		
1. Exudative tonsillitis	4	3
2. Peritonsillar cellulitis	1	2
3. Otitis media	1	0
Non-suppurative:		
Rheumatic fever	2	5

three peritonsillar abscesses required incision and drainage, and in one instance of acute otitis media spontaneous rupture occurred. All three of these patients from the control group received penicillin as therapy. Symptoms of otitis media were present in six untreated and four treated patients but were not severe enough to require specific therapy. In the group treated with cortisone, one patient developed acute appendicitis, the symptoms of which began 20 hours after the initial injection. With continued administration of cortisone, abdominal tenderness and rigidity, increase in pulse rate, and an elevated leucocyte count persisted. At operation, 20 hours after the onset of abdominal symptoms, a gangrenous appendix was removed. No *beta*-hemolytic streptococci were isolated from cultures of the appendix taken at the time of operation.

Suppurative complications, which became evident within ten days after the period of hospital observation, occurred in six patients in the treated group and in five in the control group. In the group receiving cortisone four patients with recurrences of exudative tonsillitis, one with peritonsillar cellulitis and one with acute otitis media were readmitted to the hospital. In the control group, there were readmissions for exudative tonsillitis in three cases and peritonsillar cellulitis in two patients.

A total of seven patients developed a non-suppurative sequela diagnosed as definite rheumatic fever according to a modification (10) of the classification of Jones (11). Two of the patients with rheumatic fever had received cortisone and five had received no therapy. All seven patients exhibited migrating polyarthritis, fever, and an elevated sedimentation rate. A past history of rheumatic fever was obtained in only one patient who was from the control group. The electrocardiographic abnormalities are recorded in Table III. The interval from the onset of the observed respiratory infection to the first symptom of rheumatic fever was 18 and 27 days in the treated group and 13, 19, 19, 27 and 28 days in the control patients. None of the seven patients showed bacteriological evidence of an intervening streptococcal infection, although one patient from the control group (interval 13 days) entered the hospital with peritonsillar cellulitis four days before the first symptom of rheumatic fever.

There were 15 patients who exhibited abnormalities of the P-R interval on the electrocardiogram recorded about 21 days after the onset of exudative tonsillitis or pharyngitis (Table III). Of the patients who received cortisone the P-R interval was greater than 0.21 seconds in five, and only one of these had symptoms of rheumatic fever. In the control group of patients four showed a prolongation of the P-R interval, three of whom had symptoms of rheumatic fever. In addition, there were three patients in the treated and three patients in the control group whose P-R interval increased at least 0.04 second on the 21st day as compared to the initial recording.

Of the group of eight patients who showed abnormal P-R intervals on the electrocardiogram and who showed no other evidence of rheumatic fever, four in the cortisone treated group showed bacteriological evidence of a new infection. A new type of streptococcus was isolated from the phar-

TABLE III

Effect of cortisone therapy of acute streptococcal infections on the post-streptococcal electrocardiogram

Classification	Cortisone group		Control group	
	Total cases	Cases	Total cases	Cases
Rheumatic fever:	2		5	
P-R interval greater than 0.21 second		1		3
Increase P-R interval of at least 0.04 second*		1		2
No symptoms of rheumatic fever:	85		82	
P-R interval greater than 0.21 second		4		1
Increase in P-R of at least 0.04 second*		2		1
Totals	87	8	87	7

* Exclusive of individuals showing a P-R interval of over 0.21 second.

ynx of three patients at the time of the second electrocardiogram but there was no other evidence of infection. In one patient a new type of streptococcus was isolated at the time of readmission to the hospital for exudative tonsillitis seven days after the onset of the original infection.

Total leucocyte counts were obtained daily during hospitalization in 96.6 per cent of the cases. The percentage of leucocyte counts greater than 10,000 is recorded in Table IV according to hospital day. The number of elevated leucocyte counts fell continuously in the control group. In contrast, the treated group showed a decrease in elevated counts on the second day followed by a return to a high level and then a gradual fall.

Group A streptococci were isolated at some time during hospitalization in 95.3 per cent of the cortisone treated group and 96.5 per cent of the control group. The same type of organism was

TABLE IV

Effect of cortisone therapy in streptococcal sore throat on the total leucocyte count

Group	Per cent of leucocyte counts greater than 10,000 according to hospital day				
	1	2	3	4	5
Cortisone	95.1	78.7	90.7	83.5	78.8
Control	92.9	83.5	54.7	48.2	48.1

isolated during the acute illness and throughout the follow-up period in 79.5 per cent of the treated group and 71.6 per cent of the untreated group. A new type of streptococcus was identified in 8.5 per cent of the cortisone group and 4.9 per cent of the control group. All of these changes in type, except for one instance in the control group, were demonstrated after discharge from the hospital.

The antistreptolysin titers of the sera collected on admission to the hospital and five to seven days after the onset of the disease are recorded in Figure 4. Since the distribution of the antistreptolysin titers of the acute phase sera is very similar in the two the average rise in the antibody titer during convalescence may be compared.

At the end of the first week of illness the sera of the control group showed an average rise of 0.770 dilutions (Table V) as compared to a rise of 0.541 in the cortisone treated patients. During

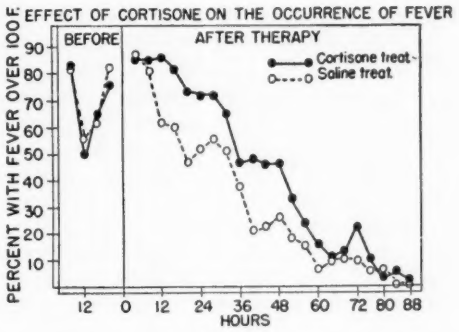

FIG. 3

the subsequent three weeks the average increase in antibody was somewhat greater in the treated group than in the patients who received no therapy. These differences were 0.175, 0.181, and 0.332 dilution increments at the second, third and fourth weeks, respectively.[5]

[5] In the treated series, those patients who gave a history of tonsillectomy had a higher antistreptolysin response than those with no such history. The average dilution increment in those without tonsils at the end of two, three and four weeks was 4.454, 5.173, and 5.173, respectively, as compared to 3.350, 3.650, and 3.542 in the patients with tonsils. No difference was noted in the control group.

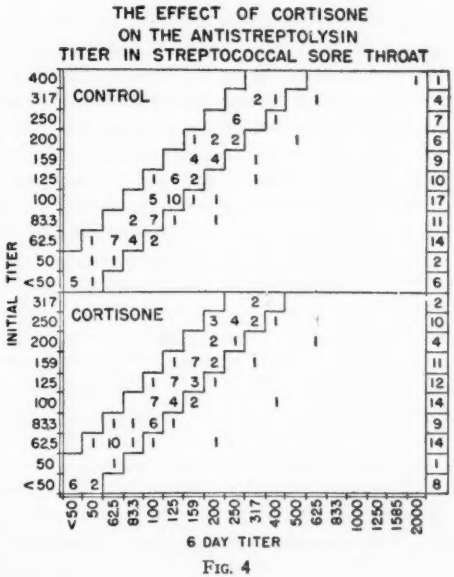

FIG. 4

TABLE V

*The effect of cortisone on the antistreptolysin titer**

Group	Days after onset of illness	Number of sera	Average dilution increase in antistreptolysin titer†
Cortisone	5–7	84	0.541
Control		84	0.770
Cortisone	14–18	79	3.607
Control		81	3.432
Cortisone	20–25	78	4.012
Control		83	3.831
Cortisone	27–32	75	3.973
Control		78	3.641

* The three patients who received penicillin therapy are excluded.

† The initial dilutions of sera employed in the tests are recorded in Figure 4. Each change in titer of one dilution is assigned a value of one (plus or minus). All changes in titer are related back to the titer of the serum specimen obtained during the first 31 hours of illness.

The only untoward effect noted during cortisone therapy was soreness around the area of injection in all patients. The soreness was most marked during the first 48 hours of therapy and diminished somewhat by the time of the last injection.

DISCUSSION

Adrenocorticotrophic hormone (ACTH) and adrenal cortical extracts have been used in studies designed to determine the effect of these hormones on acute bacterial infections. Perla and Marmorston (12) treated 17 cases of bronchopneumonia, one case of induced malaria, and six severe "grippal" infections with adrenal cortical extract. These authors report that such therapy was beneficial in that it shortened the convalescent period. Jacobs (13) treated non-immunized children who had whooping cough with adrenal cortical extract and reported the mildest course in this group when compared to other similar groups receiving pertussis antigens or triple typhoid vaccine. More recently, Finland, Kass, and Ingbar (14) used ACTH in the treatment of one case of pneumococcal pneumonia and one patient with virus pneumonia. These patients showed rapid symptomatic improvement.

In contrast to the above studies, the data presented here show that the adrenal hormone, cortisone, in the doses used, had no effect on the symptoms or physical signs of acute streptococcal sore throat. Furthermore, fever appeared to be prolonged in those patients receiving cortisone.

Whether cortisone therapy affects the development of complications can not be determined from the data obtained. There were ten such complications which developed in the control group during the period of treatment with saline and five in the cortisone treated group. There was one patient treated with cortisone who exhibited abdominal tenderness and rigidity, rapid pulse rate, fever and leucocytosis. This is in contrast to the report of Beck, Browne, Johnson, Kennedy and Mackenzie (15) who observed two patients who developed peritonitis during treatment with ACTH but who failed to exhibit the classical features of this disease.

Leucocytosis during the course of cortisone therapy as demonstrated in this study has been noted previously (16–19). The decrease in percentage of treated patients having leucocyte counts greater than 10,000 on the second day (Table IV) can possibly be explained by the fact that on this day most of the determinations were made about one hour after treatment. This drop in total leucocyte count immediately following the injection of ACTH and adrenal cortical extracts has been described (1, 20, 21). The remaining counts were taken about 13 hours following the previous injection.

A number of investigators have studied the relationship between adrenal hormones (22–30) and antibody production. Chase, White, and Dougherty (22) report that the administration of adrenal cortical extracts to animals receiving injections of staphylococcal toxin, horse serum, egg albumin or sheep cells results in higher antibody titers than obtained in control animals which received no adrenal extract. Mirick (23), however, reports that in man the administration of ACTH or cortisone does not alter the antibody response to immunization with pneumococcal polysaccharides. Finland, Kass, and Ingbar (14) observed that in one patient with pneumococcal pneumonia treated with ACTH antibodies appeared in the serum at about the expected time.

In contrast to the above reports, Fischel (24) demonstrated an inhibition of antibody formation in rabbits given ACTH during immunization with

formalinized pneumococci. Germuth and Ottinger (25) report that ACTH and cortisone administration markedly suppressed the formation of antibody to egg albumin in rabbits. In these experiments 10 mgms of cortisone were administered daily for 18 days to rabbits weighing 2 kgms. Such therapy completely inhibited antibody formation to egg albumin. It is to be noted, however, that antibody was not measured several weeks after the experimental period was completed. The relative doses of cortisone employed in these rabbits were greater than those used in the present study.

Antibodies to various products of group A streptococci are especially high in patients with rheumatic fever (31, 32). Recently it has been demonstrated that the attack rate of rheumatic fever following streptococcal pharyngitis is related, in part, to the magnitude of the antibody response (33). Procedures which alter this antibody response may also alter the incidence of subsequent rheumatic fever. Thus penicillin therapy of acute streptococcal pharyngitis inhibits the formation of antibody presumably because of the eradication of the antigen (streptococci) from the throat and at the same time such treatment markedly decreases the incidence of acute rheumatic fever (10).

Cortisone therapy as employed in the present study failed to alter appreciably the antistreptolysin response to streptococcal infections. Because the sera of the treated group showed a somewhat lower antibody titer at six days than the sera of the control group there may have been a slight inhibition of antibody formation. It should be emphasized, however, that the increase in antibody was somewhat greater in the treated group than in the control patients two, three and four weeks after the onset of illness. This may be due to the fact that cortisone is not antibacterial, so that when treatment was discontinued the antigenic stimulus was still effective.

The effect of cortisone therapy on acute streptococcal infections is of especial interest not only because of the above considerations, but also because the compound also exerts a remarkable effect when administered to patients with rheumatic fever (2–4). Acute rheumatic fever was not prevented by cortisone therapy. Although there were only two cases in the treated group and five cases among the control patients, this distribution could have occurred by chance. Since the incidence of abnormal electrocardiograms was equal in the treated and control group of patients, cortisone therapy had no effect on these signs which are frequently associated with rheumatic fever. Since there may have been a slight inhibition of antibody formation during the period of therapy, it is entirely possible that larger doses of cortisone administered for a longer period of time may inhibit antibody production and reduce the incidence of rheumatic fever.

SUMMARY

Eighty-seven treated and 87 control patients were studied to determine the effect of cortisone on the clinical and immunological response to streptococcal respiratory infections. Cortisone therapy, in the dosages employed, exerted no effect on the symptoms or physical signs of this infection. Patients who received cortisone exhibited fever for a longer period of time than the control patients. Suppurative complications developed during the period of cortisone or saline injections in one of the treated patients and in three of the control subjects.

Rheumatic fever was diagnosed in two treated and five control subjects. The P-R interval recorded approximately three weeks after the onset of illness was greater than 0.21 second in a total of five of the treated and four of the control group.

The increase in the antistreptolysin titer was somewhat less in the sera of the treated group than in the control patients one week after the onset of the infection, but at two, three and four weeks the formation of antibody was slightly greater in treated than in the control patients.

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THE MECHANISM OF POLYURIA OF DIABETES INSIPIDUS IN MAN. THE EFFECT OF OSMOTIC LOADING

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INTRODUCTION

Although there is general agreement on the role of the hypothalamic-hypophyseal region in the genesis of diabetes insipidus (1), the nature of the renal defect which results in the excretion of a copious and dilute urine has remained obscure. The excretion of a hypotonic urine in diabetes insipidus may be explained by any of three hypotheses: (*a*) the delivery of a hypotonic fluid from the proximal tubule, or (*b*) an isotonic proximal urine with solute reabsorption in the distal tubule, and (*c*) an isotonic proximal urine with water secretion in the distal tubule.

A complete decision with regard to these hypotheses is not at hand. Hypothesis (*a*), assuming a hypotonic proximal fluid (2), although presumably based on the observations of Walker and his associates (3) would require a much greater hypotonicity than ever observed in order to explain the low urinary osmolarity of diabetes insipidus. Hypothesis (*b*) (4), most widely accepted at present, holds that the polyuria is due to lack of water reabsorption in the distal renal tubule. Normally $\frac{7}{8}$ of the glomerular filtrate is reabsorbed isotonically in the proximal tubule, while $\frac{1}{8}$, under the hormonal control of the hypothalamic-hypophyseal region, may be reabsorbed in the distal tubule. Lack of hormone results in a maximum diuresis of about $\frac{1}{8}$ of the glomerular filtration rate. This hypothesis, in order to explain the dilute urine of diabetes insipidus, requires the assumption of solute reabsorption in the distal tubule, with a distal reabsorbate of unspecified but hypertonic osmolarity. Hypothesis (*c*), popular before the general acceptance of the glomerular filtration theory, has for its major objection the demonstrated proportionality between urine flow and arterial pressure in the isolated kidney (5).

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It appeared that osmotic diuresis in patients with diabetes insipidus might offer a means of testing the three hypotheses. Given an unchanged glomerular filtration rate, and assuming with Shannon (2) that the urine formed in diabetes insipidus resembles that which is delivered to the distal tubule, no significant change in urinary osmolarity should occur after loading. The predictions of hypothesis (*b*) depend on the nature of the distal reabsorptive process assumed. Hypothesis (*c*) would require that, given a constant rate of secretion of hypotonic urine prior to loading, the increment of urine flow during osmotic diuresis, representing additional proximal fluid, be nearly isotonic with plasma.

Osmotic diuresis with mannitol loading was produced in three subjects with diabetes insipidus. The rates of urine flow and solute excretion before and during loading as well as those of glomerular filtration and renal plasma flow were studied.

EXPERIMENTAL

The subjects of the present study were one woman, 19 years, and two boys, six and 12 years of age, with diabetes insipidus. They had abstained from food and drink for four to eight hours, and from pitressin medication for two to three days preceding the test.

Mannitol, in 25% solution, was administered as the loading solute. Two priming injections, the first of 180 and the second of 90 milliosmoles (mOsm.) per 1.73 M^2, calculated to add 20 and 10 mOsm., respectively, per liter of distribution volume, were given, followed by a maintenance infusion in the amount of 1% of the priming dose per minute. All infusion fluids had sufficient para-aminohippurate (PAH) added to maintain a plasma level of about 3 mgm./100 cc., for the measurement of renal plasma flow. After a 20 minute period of equilibration following the priming infusion, urine was collected for two or three periods of 15–20 minutes each at both loading levels and blood samples were drawn at appropriate intervals. The urine and blood were analysed for mannitol, urea, sodium, potassium, chloride, and PAH. Osmotic activity was determined directly by the freezing

point in urine, and calculated from the determined [2] solutes in blood. The analytical techniques used have been described (6).

Toward the end of the test periods, the patients became weak and listless and the skin felt cold to touch. One patient, D. S., complained of headache, and another vomited repeatedly when he drank water after the test.

The tests were repeated on two of the patients, D. E. and I. W., after an interval of three to four weeks, while they were receiving pitressin. Five-tenths cc. of an aqueous solution of pitressin (10 pressor units) was given intramuscularly every three hours for 20 hours preceding, and every two hours during the test. On pitressin, the patients were able to withstand 16 hours of water deprivation, and experienced no untoward symptoms during the loading procedure.

[2] Osmotic Activity of Plasma $= [2(Na + K) - 8 + Urea + Mannitol] \times \gamma$, where γ, the activity coefficient of plasma, was assumed to be 0.92.

RESULTS

Table I presents results on three patients obtained in five tests comprising 40 periods of urine collection. Data include the corrected minute volume of urine, the osmolarity of urine and plasma, clearance of mannitol and PAH, and the filtration fraction.

It may be seen that during the pre-loading periods, the urine flow remained at high values of 5.1–11 cc./min., despite the hydropenic state of the subjects. The urine was hypotonic, its osmolarity reaching values as low as 56 mOsm./L., or approximately ⅙ that of plasma. After loading, with increasing flows, the osmolarity of the urine increased. The highest value obtained, 232 mOsm./L., was slightly more than ⅔ of the plasma

TABLE I

Urine flow, osmolarity, and clearances of mannitol and PAH during mannitol diuresis

Period no.	Concurrent time	Urine volume	Osmolarity urine	Osmolarity plasma	Clearance mannitol	Clearance PAH	Clearance mann. / Clearance PAH
	min.	*cc./min./1.73 M²*	*mOsm./L.*	*mOsm./L.*	*cc./min./1.73 M²*	*cc./min./1.73 M²*	
I. W., 2-25-50, S.A. 1.45 M², Mannitol, No pitressin:							
P-1	−76 to −46	5.14	67				
P-2	−46 to −18	6.76	58				
P-3	−18 to −6	7.31	56	285			
	0 to 7.0		121 cc. of 25% Mannitol				
X-I	−6 to 20	14.0	124				
1	20 to 30	15.5	158	291	104.2	498	0.21
2	30 to 40	14.4	155	295	92.2	451	0.20
	42 to 50		104 cc.of 25% Mannitol				
X-II	40 to 62	19.6	185				
3	62 to 80	17.6	208	300	90.8	496	0.18
4	80 to 90	14.9	228	304	84.9	472	0.18
5	90 to 102	14.5	232				
D. E., 12-8-49, S. A. 0.81 M², Mannitol, No pitressin:							
P-1	−71 to −32	6.92	132				
P-2	−32 to −10	7.58	113	300			
	0 to 10		69 cc. of 25% Mannitol				
X-I	−10 to 21	11.8	159				
1	21 to 40	11.3	170	316	54.5	325	0.17
2	40 to 58	10.7	184	317	52.0	329	0.16
	60 to 69		36 cc. of 25% Mannitol				
X-II	58 to 75	14.7	195				
3	75 to 93	15.2	200	324	55.4	388	0.14
4	93 to 125	11.0	212	332	43.9	256	0.17

TABLE I—*Continued*

D. S., 2–6–50, S. A. 0.83 M², *Mannitol, No pitressin:*

Period no.	Concurrent time	Urine volume	Osmolarity urine	Osmolarity plasma	Clearance mannitol	Clearance PAH	Clearance mann. / Clearance PAH
	min.	*cc./min./1.73 M²*	*mOsm./L.*	*mOsm./L.*	*cc./min./1.73 M²*	*cc./min./1.73 M²*	
P-1	−69 to −37	10.7	56				
	0 to 10		71 cc. of 25% Mannitol I.V.				
X-I	−37 to 16	13.1	79				
1	16 to 26	27.9	130				
2	26 to 37	18.8	135				
	39 to 49		37 cc. of 25% Mannitol				
X-II	37 to 50	19.8	147				
3	50 to 59	21.0	146				
D. E., 1–11–50, S.A. 0.82 M², *Mannitol, Pitressin:*							
P-1	−94 to −46	1.15	777				
P-2	−46 to −12	1.52	747	263			
	0 to 7.4		61 cc. of 25% Mannitol				
X-I	−12 to 35	5.91	583				
1	35 to 60	6.50	605	269	145.5	597	0.24
2	60 to 78	6.18	602	271	135.2	554	0.24
	79 to 84		40 cc. of 25% Mannitol				
X-II	78 to 102	8.29	551				
3	102 to 130	8.31	538	277	118.5	513	0.23
4	130 to 157	8.84	511	277	132.8	469	0.28
I. W., 3–25–50, S.A. 1.45 M², *Mannitol, Pitressin:*							
P-1	−73 to −40	0.43	605				
P-2	−40 to −3	0.76	677	303			
	0 to 11		121 cc. of 25% Mannitol				
X-I	−3 to 25	3.50	606				
1	25 to 35	3.62	606	307	95.6	565	0.17
2	35 to 54	4.15	611	307	107	606	0.18
	58 to 69		104 cc. of 25% Mannitol				
X-II	54 to 79	4.83	624				
3	79 to 89	5.33	608	314	95.5	405	0.24
4	89 to 99	6.45	605	318	104	510	0.20

osmolarity. Urine flows increased two- to threefold over the preliminary rates to values of 15–28 cc./min. These effects are the reverse of those observed in normal hydropenic subjects during solute loading, where with increasing flows urinary osmolarity decreased but remained greater than that of plasma (6).

The last two tests shown in the table were performed on patients D. E. and I. W. while they were under the influence of pitressin. During the pre-loading periods, the urine flow varied between 0.43 to 1.15 cc./min.[3] After loading, with flows attaining values as high as 8.8 cc./min., urinary

[3] The high preliminary flows observed in patient D. E. perhaps may be explained by a mild electrolyturetic action of the pitressin.

osmolarity decreased to a value of 511 mOsm./L. The results were essentially the same as those observed in normal hydropenic subjects after mannitol loading (6).

The clearances of mannitol and PAH were low in patient D. E., a 12 year old dwarfed boy. When the test was repeated with the patient on pitressin, the clearance of mannitol increased three-fold and that of PAH, two-fold, with an increase in the filtration fraction. Decreases in filtration rate have been observed in dogs with diabetes insipidus (7). In the case of patient I. W., the clearances of both mannitol and PAH were normal and did not change significantly after pitressin administration.

It is evident that in each patient, the clearance values remained unchanged during the tests. Although no clearances were determined in the preliminary periods, one may assume that they changed little with loading both from the constancy of the clearances at different levels of loading and from data in the literature on normal subjects and dogs (8).

The independence of urine flow from changes of filtration rate or renal plasma flow is evident from the data shown in the table.

Figure 1 contains a plot of the values for urine flow versus solute load. The curve drawn is based on the data of nine normal hydropenic subjects during mannitol diuresis. It can be seen that for a given solute load, the patients with diabetes insipidus excreted urine at a rate two to three times greater than normal subjects. On the other hand, when treated with pitressin, their response was normal. A dependence of urinary volume on the "total milliosmolar excretion rate" in patients with diabetes insipidus has been noted (9). It is of interest that both patients on pitressin excreted solutes in an identical manner despite sizeable differences in their filtration rate.

Figure 2 shows plots of losses of sodium, chlo-

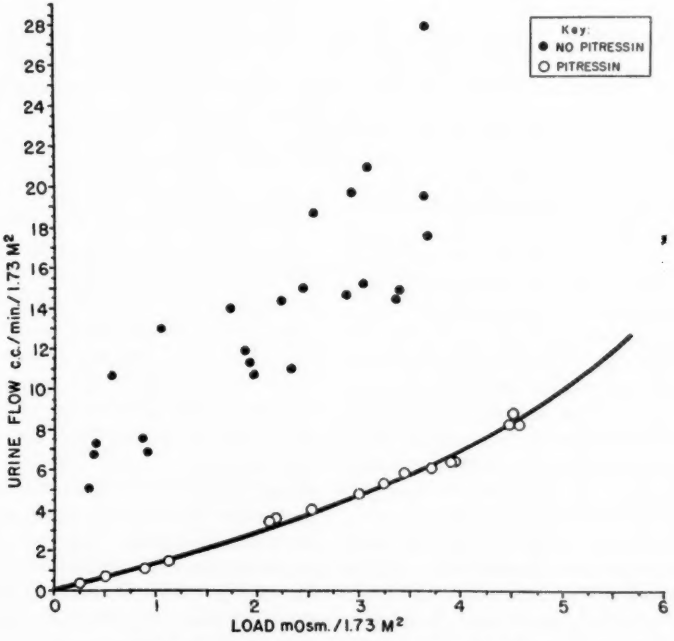

FIG. 1. URINE FLOW VERSUS OSMOTIC LOAD

Open and solid circles refer to the patients with diabetes insipidus, with and without pitressin therapy, respectively. The line represents the average flow-load curve of nine normal subjects.

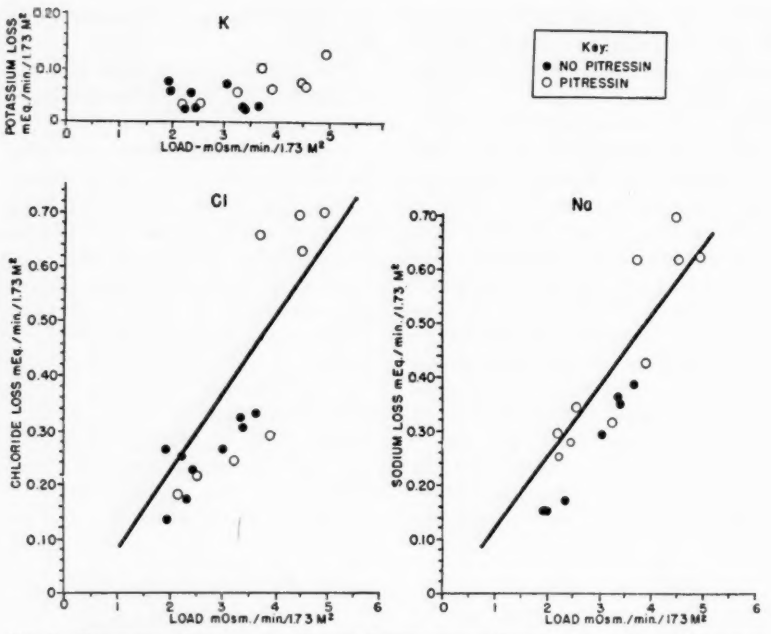

FIG. 2. POTASSIUM, CHLORIDE AND SODIUM LOSSES VERSUS OSMOTIC LOAD DURING MANNITOL LOADING

The symbols have the same meaning as in Figure 1.

ride, and potassium versus total solute load. The lines represent linear regressions calculated from nine normal hydropenic subjects under conditions of osmotic loading with mannitol. It can be seen that the data group themselves about the lines in a fairly satisfactory manner. The pattern of salt loss was the same in both pitressin-treated and untreated patients, despite the wide disparity in urine flows. Solute loading had little effect on potassium excretion in the subjects of the present study as observed previously in normal subjects (10). Mannitol excretion, not plotted, accounted for the same percentage of urinary solutes (50–60%) as it did in normal subjects (6).

The linear relation of sodium, chloride, and mannitol to the total solute load indicates that the per cent composition of the urine in patients with diabetes insipidus during solute loading is similar to that of normal subjects. It also suggests that the final solute load destined for excretion is assembled in a portion of the tubule proximal to the site of concentration or dilution of the urine.

DISCUSSION

The observed two- to four-fold increase of urinary osmolarity in patients with diabetes insipidus after osmotic loading appears incompatible with a hypothesis which requires the delivery of a hypotonic fluid from the proximal tubule. The converse hypothesis, namely, the flow of an isotonic proximal fluid, may be tested, given the additional assumption that there exists a basal, dilute urine flow, that observed in the preliminary periods, which changes little throughout the experiment. After loading, the increment of urinary volume and load above the control level should consist of isotonic proximal fluid. This may be calculated as the increment of urinary load divided by the plasma osmolarity. Table II contains the calculations of the urine flow based on these assumptions, compared with the observed values. The data represent 23 periods of urine collection, of which 18 were obtained during loading. It can be seen that the calculated and observed rates of flow were in

TABLE II

Urine flow calculated on the assumption of constant basal flow and addition of isotonic proximal fluid

Patient	Period	(a) Load	(b) Assumed load added*	(c) Plasma osmotic activity	(d) Calculated values: Increment prox. fluid (b + c)	(e) Calculated values: Urine† flow	(f) Observed flow urine
		mOsm./min.	*mOsm./min.*	*mOsm./L.*	*cc./min.*	*cc./min.*	*cc./min.*
D. E.	P-1	0.92		300			6.9
	P-2	0.86					7.6
	X-I	1.88	0.99		3.14	10.4	11.8
	1	1.93	1.04	316	3.29	10.5	11.3
	2	1.97	1.08	317	3.42	10.7	10.7
	X-II	2.87	1.98		6.15	13.4	14.7
	3	3.04	2.15	329	6.67	13.9	15.2
	4	2.34	1.45	332	4.37	11.6	11.0
I. W.	P-2	0.39		285			6.8
	P-3	0.41					7.3
	X-I	1.74	1.34		4.57	11.6	14.0
	1	2.45	2.05	291	6.99	14.0	15.5
	2	2.23	1.83	295	6.24	13.3	14.4
	X-II	3.63	3.23		10.7	17.7	19.6
	3	3.66	3.26	300	10.8	17.8	17.6
	4	3.40	3.00	304	9.93	17.0	14.9
	5	3.36	2.96		9.80	16.8	14.5
D. S.	P-1	0.60					10.7
	X-I	1.04	0.44		1.56	12.3	13.1
	1	3.63	3.60	282	12.8	23.5	27.4
	2	2.54	1.94		6.88	17.6	18.8
	X-II	2.92	2.32		7.95	18.6	19.9
	3	3.07	2.47	292	8.46	19.2	21.0

Columns (a) and (b) present the values for the observed urinary load and its increment during loading; and column (c), the values for the osmotic activity of the plasma. The calculated increments of proximal fluid, *i.e.*, the increment of load divided by plasma osmolarity, are presented under column (d); calculated values for the urine flow, obtained by adding the calculated increment of isotonic fluid to the basal urine flow, under column (e); and the values for the observed urine flow, under column (f).

* The assumed load added is the difference between post-loading and average pre-loading solute loads.

† Sum of increment of proximal fluid (d) and basal flow, taken as the average of pre-loading flows.

reasonably close agreement, the maximum deviation being 17%. The close agreement between theoretical and observed urine flows supports the validity of both initial assumptions, *i.e.*, an unchanging basal flow and addition of isotonic fluid from the proximal tubule during loading. Since the method of calculation is independent of the nature of the basal urinary flow and load, the predictions hold for a hypothesis of solute reabsorption, if a constant rate of reabsorption [4] in the distal tubule both before and after loading is assumed, as well as for a hypothesis of distal secretion of water.

Direct proof for either hypothesis is not yet available. Much indirect evidence, martialled from phylogenetic, physiologic, and physico-chemical considerations, favors a process of a distal secretion rather than one of reabsorption.

That tubular secretion occurs in the aglomerular kidney of certain marine teleosts has been shown by Marshall (11), who found, in the absence of any filtration process, production of a urine isotonic with or hypotonic to the plasma. Bieter (12) added further evidence by showing that the secretion pressure in the ureter was greater than the blood pressure in the dorsal aorta. There is no *a priori* phylogenetic reason to deny the existence of a secretory function for water in higher animals with glomerular kidneys.

Data from studies on water diuresis in man or dog (13–16) may be interpreted in terms of tubular secretory activity. The ingestion of water is followed by a characteristic response in urine flow. After a brief lag period the urine flow increases to a maximum and declines sharply to the initial

[4] The assumption of a variable rate of distal solute reabsorption would fail to explain the added flow or load observed during loading.

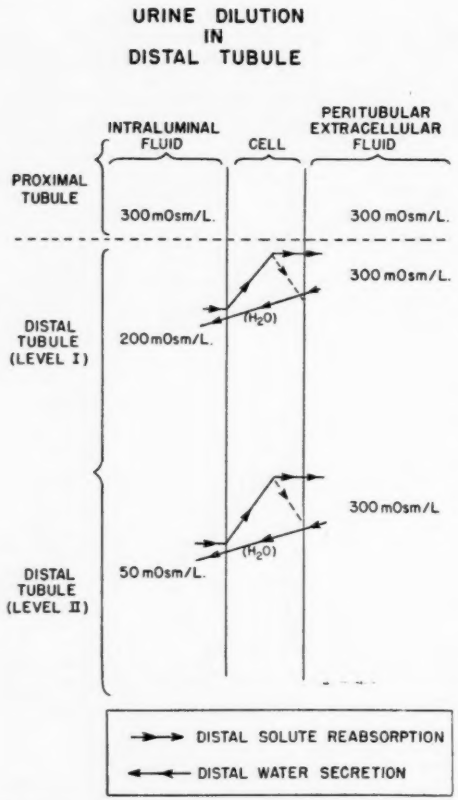

FIG. 3. SCHEMATIC COMPARISON OF SECRETORY AND REABSORPTIVE PROCESSES OF URINE DILUTION IN THE DISTAL TUBULE

It is assumed that the proximal and peri-tubular extracellular fluids are both isotonic with plasma, but hypertonic to the distal fluid. The assumed osmolarities are designated in the diagram. The tonicity of the final urine has been taken as ⅙ that of plasma. The lines pointing toward the left represent energy potentials for the process of secretion with the production of a fluid approaching water in composition. The lines pointing to the right represent energy potentials for an assumed process of distal solute reabsorption which requires a hypertonic reabsorbate. Two levels of the distal tubule, representing progressive stages of urine dilution, are portrayed. No definite value is assumed for the tonicity within the cell for the reabsorptive process. Alternative pathways from the cell to the extracellular fluid are drawn for the reabsorbate, solid lines representing a process of extrusion of a hypertonic fluid, and interrupted ones, a process of intracellular dilution.

level. Variations in the amount or in the mode of administration of water do not affect the pattern or extent of the diuresis, provided the dose is in excess of 1% of the body weight (15*b*). Even while water absorption in the intestine or injection by vein still continues (16*b*, 17), the typical decline of diuresis takes place. Throughout the entire course of water diuresis, the filtration rate remains essentially unchanged (18–20). Hormonal and nervous factors, *i.e.*, pituitrin, epinephrine, thyroxine, and painful or emotional stimuli, have profound effects on both the course and amount of the diuresis (21, 22).

This behavior resembles that of secretory glands. Their function is characterized by a specific pattern often rhythmical in nature (23, 24). The volume of secretion may vary independently of the blood flow (25). Nervous and hormonal factors profoundly affect their activity. Hypotonicity of secretions is well known, sweat and saliva being the outstanding examples (26).

Some of the thermodynamic implications of the production of hypotonic urine, according to the alternate assumptions of distal secretion of water or distal reabsorption of solutes, may be examined. Figure 3 is a schematic presentation of the assumed energy relations and fluid concentrations at two levels of the distal tubule. At any level in the distal tubule the concentration gradient between peri-tubular fluid and the secretion is 300 mOsm./L., while that between intra-luminal and peri-tubular fluids increases progressively. With respect to an assumed process of solute reabsorption, the less the volume of water reabsorbed, the more hypertonic must be the reabsorbate. No specific assumptions are available for the volume of fluid delivered to or reabsorbed from the distal tubule. For simplicity, a constant rate of solute reabsorption at all levels in the distal tubule has been assumed. Whatever volume of a hypertonic reabsorbate is postulated, its production will require more energy than that of a dilute secretion.[5]

[5] The energy expenditure in the production of dilute urine by the distal tubule, assuming a single stage physical process, may be calculated according to the equation of von Rhorer (27).

$$1)\quad W = VRT\,(U \ln U/P + P - U)$$

The limitations of and justifications for such a method of calculation of osmotic work have been discussed in detail elsewhere (10).

The volume of water secreted or reabsorbate removed to

Inspection of the diagram reveals that the concentration gradient between lumen and reabsorbate at any level in the tubule is greater than that between peri-tubular fluid and a dilute secretion. Furthermore, the gradient increases progressively as the urine becomes more dilute. Whether the hypertonic reabsorbate *per se* is extruded into the peri-tubular space or whether it is diluted to isotonicity in the tubular cell, the process of distal solute reabsorption would seem uneconomical compared to that of distal secretion.

According to the postulate that there is a distal secretion, water is transported from an isotonic peri-tubular fluid to the tubular lumen. Such a process may be experimentally produced and is known as "negative anomalous osmosis." The factors responsible for negative osmosis depend chiefly on the characteristics of the membrane (28). A similar process of negative osmosis might result in movement of water from hypertonic distal to isotonic peri-tubular fluids, accounting for the concentration of urine in normal hydropenic subjects or in patients with diabetes insipidus when they receive pitressin. No fundamental change in membrane permeability would have to be assumed to account for either concentration or dilution of the urine by the kidney.

The large volume of glomerular filtrate renders difficult any attempt to establish the existence of a tubular secretory function. Perhaps the only acceptable direct evidence would consist of demonstration of a rate of urine flow in excess of that of filtration, under conditions of extreme reduction of filtration rate.

dilute a given amount of isotonic proximal fluid may be calculated as follows:

It is easily seen that

$$2)\quad (O_p - O_u)n$$

is the number of solutes removed in the process of formation of dilute urine from an isotonic fluid, be it peri-tubular, extracellular or proximal tubular fluid, where O_p = plasma osmolarity, O_u urine osmolarity, and n the observed rate of urine flow. Then,

$$3)\quad \frac{(O_p - O_u)n}{O_p} = V_s,$$

the volume of a secretion of water added to a given amount of isotonic proximal fluid, and

$$4)\quad \frac{(O_p - O_u)n}{O_r} = V_r,$$

the volume of a reabsorbate of osmolarity O_r, removed from a given amount of isotonic proximal fluid.

It follows that,

$$5)\quad V_r = \left(\frac{O_p}{O_r}\right) V_s.$$

In the case of distal secretion, the concentration gradient is from peri-tubular to secretion fluid. Substituting in equation *1*), U is the concentration of the secretion, and P that of the peri-tubular fluid. In the case of solute reabsorption, the gradient is from distal fluid to reabsorbate, so that U would represent the assumed osmolarity of reabsorbate and P, that of the distal fluid. As a first approximation, the osmolarity of the secretion may be taken as zero. For the reabsorbate, a series of values in multiples of plasma osmolarity are assumed. The osmolarity of the peri-tubular fluid is taken to be 300, and the final urine, 60 mOsm./L. Substituting these values in equation *1*) gives a series of relative work units which permit a comparison of energy expenditure in terms of a given volume of isotonic fluid cleared of solutes as is shown in the following table:

TABLE III

Energy expenditure for secretory and reabsorptive process in the distal tubule

Vol. of secretion	Osmol. peri-tub. fluid	Osmol. of secretion	Work units
cc./min.	*m Osm./L.*	*m Osm./L.*	*W/RT*
V_s	300	0	300 V_s
Vol. reabsorbate	Osmol. distal fluid	Osmol. reabsorbate	
$V_s/2$	60	600	420 V_s
$V_s/3$	60	900	536 V_s
$V_s/4$	60	1,200	615 V_s
$V_s/10$	60	3,000	876 V_s

SUMMARY

Osmotic diuresis was produced by mannitol loading in three patients with diabetes insipidus.

During hydropenia, a flow of copious and dilute urine persisted. After loading, osmolarity of the urine rose but remained below that of plasma while the urine flow increased more than two-fold. For a given solute load the rate of excretion of urine was two to three times that of normal subjects. The urine flow was independent of changes of filtration rate or blood flow. After administration of pitressin, the relation of urine flow and solute excretion became essentially normal.

The per cent composition of urinary solutes in patients with diabetes insipidus during mannitol loading was similar to that of normal subjects suggesting that the final solute load destined for excretion is assembled in a portion of the tubule proximal to the site of concentration or dilution of the urine.

The data on urinary flow and solute load indicate that there exists a basal, dilute urine flow in diabetes insipidus, and that the increased flow ob-

served during loading is due to the delivery of additional isotonic fluid from the proximal tubule.

It is postulated, on indirect evidence, that the distal tubule may be a special organ of water transport and that the production of hypotonic urine is due to water secretion, that of a hypertonic urine to water reabsorption, in the distal tubule.

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FACTORS MAINTAINING CEREBRAL CIRCULATION DURING GRAVITATIONAL STRESS

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INTRODUCTION

The stresses developed during flight may make certain physiological mechanisms clear which would otherwise be difficult to demonstrate. This report is concerned with a condition induced by forces peculiar to high speed aircraft, but the results can be applied to the cerebral circulation during the more familiar stresses induced by gravity in the erect position. Amaurosis fugax, or aviator's blackout (1), occurs in normal men when they are exposed in the upright seated posture to centrifugal forces acting for more than five seconds in the direction head to buttocks. Commencing at 3 to 4 gravity units as a dimming of vision most noticeable in the peripheral field, at 4 to 5 gravity units it progresses to temporary blindness and finally, at slightly higher levels, unconsciousness occurs. Lambert (2) has shown that apparently identical changes in vision can be produced by air pressure on the eye balls. By sucking on the eyes with a special mask, he prevented blackout during high accelerations. His experiment confirmed the belief that the centrifugal forces induce blackout by causing a decrease in the arterial pressure at eye level. This pressure drop results from an increase in the effective weight of the blood which increases the effective length of the column above the heart. Since the pressure is proportional to the acceleration, at 5 gravity units the normal arterial pressure becomes inadequate, for it must then raise the blood the equivalent of five times the 30 cms. distance from the heart to the head. Lambert and Wood (3) have shown that the systolic pressure at eye level during exposures producing blackout is less than 20 mm. Hg, and they have observed that the pressure often falls temporarily to zero (4). Rossen, Kabat and Anderson (5) have shown that if the blood supply to the human brain is suddenly interrupted, consciousness is lost in six seconds. The circulation should, theoretically, be halted by an acceleration which reduces the mean arterial pressure at head level to near zero values. In spite of this, maintenance of consciousness has been reported during a blackout of three minutes duration on the human centrifuge (6). This continued alertness for periods of 30 seconds or more, in the face of a measured cerebral arterial pressure of 20 mm. Hg to 30 mm. Hg (7) is surprising. However, a survey of the clinical experiences with syncope also yielded extremely low values. Data from the observation of four different conditions were available (Table I), namely, clinical orthostatic hypotension (8–10); a phlebotomy followed by syncope (11); the use of a tilt table with amyl nitrite (12) and collapse following puncture of the brachial artery (13). The significant parameter in the assessment of blood flow is the mean pressure. Since this was not explicit in the clinical data, it was calculated from the systolic and diastolic pressures by adding ⅓ of the pulse pressure to the diastolic pressure. (Table I, Column M). To make these figures comparable with the data obtained on the centrifuge mean pressure at eye level was estimated by subtracting from it the hydrostatic pressure of the fluid column between the brain and the various measuring points (Table I, Column E). The recorded values for visual symptoms, ranged about 30 mm. Hg to 40 mm. Hg; those for syncope were approximately 20 mm. Hg.

These facts suggest that compensating factors are at work and that a study of cerebral circulatory competence during exposure to gravitational fields should be of interest. The nitrous oxide technique for the accurate measurement of cerebral

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blood flow in man requires a longer period than was available for applying the stress with the human centrifuge. However, since there is little storage capacity for oxygen in the small blood content of the brain tissue itself, changes of cerebral venous oxygen content will reflect circulatory changes occurring within relatively short time intervals. Kety and his associates (14) have pointed out that the reciprocal of the cerebral arterio-venous oxygen difference represents the ratio of cerebral blood flow to metabolic demand. Although it yields no information concerning either of these parameters separately, the cerebral A-V O_2 does constitute a measure of the sufficiency of the cerebral circulation. It was, therefore, decided to measure the cerebral venous and arterial oxygen saturation during acceleration.

METHODS

Arterial pressures at head level were determined by a strain gauge blood pressure transmitter (15) connected by rigid walled, incollapsible vinylite tubing to a Lindeman type cannula placed in the radial artery (16). The wrist was held at head level and the gauge was firmly attached to a cap held at eye level. Thus even if the subject slumped in his seat during the acceleration, there would be no movement between the gauge and the brain. There is only 5 mm. Hg difference between the mean pressures in the carotid and radial arteries of a supine man (17); therefore, it was felt that the pressure in the radial artery is a satisfactory measure of that in the cerebral vessels if due account is taken for hydrostatic effects. Since blood flow is a function of mean pressure, mean pressures were assessed. Planimetry of sample pulse curves confirms the accuracy of the calculations of mean pressure made by adding $\frac{1}{3}$ of the pulse pressure to the diastolic pressure (18).

The internal jugular vein was punctured by a needle inserted just below the mastoid process, using a 19 gauge needle. The technique was that introduced by Myerson, Halloran, and Hirsch (19) and described by Kety (20) in his recent account of the quantitative determination of cerebral blood flow in man. It results in the placement of the tip of the needle in a region just below the jugular foramen where collapse of the vein is impossible due to its bony attachment. A 2 by 3 cms. brass plate was fastened to the neck by adhesive tape and an attached clamp tightened around the needle to prevent slip-

TABLE I

Estimated mean cerebral blood pressure in mm. Hg during syncope

Author and Method	Cause	Case	Blood pressure (*mm. Hg*)				Symptoms as described by authors
			S/D	M*	E*	ME*	
Ellis and Haynes (8), Riva Rocci	Orthostatic hypotension	1	65/52	56	44		Feeling of faintness to absolute syncope
		2	62/46	51	29		Blurring of vision; "all gone feeling"
		3	33/26	28	6		Buzzing; blurring of vision
		4	62/41	48	24	27	Dizziness
		5	75/56	62	40		Dizziness
		6a	56/44	48	33		Marked dizziness
		6b	42/30	34	16		Marked dizziness
Jeffers, Montgomery and Burton (9), Riva Rocci	Orthostatic hypotension	WL	50/45	47	25		Vertigo; fainting ++
		LM	60/50	53	21	21	Vertigo; fainting +++
		TM	42/38	39	17		Vertigo; fainting ++++
Gregory (10), Riva Rocci	Orthostatic hypotension	1a	45/?				Fainting
		1b	50/?			23	Very dizzy
		1c	55/40	45	23		Dizzy
Loman and colleagues (12), Tycos elastic manometer, carotid artery	Sudden tilting			28	22		
	Amyl nitrite			22	16	19	Fainting
							Verge of syncope
McMicheal (11), Riva Rocci	Hemorrhage		58/?	20	<20?		Syncope
Rushmer (13), Hamilton manometer, Riva Rocci	Neurogenic collapse		65/?		<43		Dizziness
			45/?		<23		Unconsciousness

* M Mean arterial pressure obtained by adding $\frac{1}{3}$ of pulse pressure to the diastolic pressure.
E Mean arterial pressure at eye level calculated by allowing for the estimated hydrostatic pressure of the vertical column between gauge and eye.
ME Average pressure from E.

Table of data collected from various sources showing that the mean arterial pressure can fall below 30 mm. Hg before consciousness is seriously impaired, but that syncope develops at 20–25 mm. Hg.

ping during the centrifuge run. Samples could be taken rapidly during the run by a series of three-way stopcocks, to each of which was attached a syringe (20). The heparinized samples were chilled until submitted for analysis of oxygen content by the Van Slyke manometric method (21). The blood was drawn by an experimenter sitting beside the subject in the cab of the centrifuge. Runs were made at accelerations ranging from 1.0 to 4.5 g and the force was held constant for one to two minutes at each acceleration until the blood samples had been drawn. Three trained subjects were employed. Once the needles had been inserted they were at ease. Their normal resting blood pressures give objective confirmation of their relaxation. In two subjects, four series of experiments, involving prolonged acceleration, were made and the oxygen content of 13 samples of cerebral venous blood determined at the various accelerations. In three of these series the arterial blood pressure was determined at the same time. (The possibility of equipment failure with leakage of air into the jugular vein was not ignored. The dangers of a small aeroembolism are inappreciable and the observer in the cab watched the transparent tube leading to the needle. If air leakage developed the blood would disappear from the tube. This could be seen and the needle removed at once. In practice this emergency did not occur.)

Jugular pressures were recorded by a second strain gauge transmitter also held at eye level. Both systems were flushed with heparinized saline to prevent clotting. A rapid pressure rise following a Valsalva maneuver and the presence of pulsations, which persisted in spite of digital occlusion of the jugular vein during acceleration, assured the patency of the needle and its free communication with the intracranial sinuses.

In two subjects the oxygen saturation of the blood in the jugular vein was measured continuously by drawing it through a Kramer glass cuvette oximeter (22) by a mechanically actuated syringe (23). The oximeter was calibrated by passing blood of known saturation through it. Since all the samples were drawn at the same constant speed, the flow effect, characteristic of these instruments, could be disregarded.

Changes in venous oxygen saturation will only reflect the sufficiency of the cerebral blood flow provided the arterial saturation remains essentially undisturbed. Therefore, in control experiments the oximeter was connected to the arterial cannula. Arterial saturation was measured during typical centrifuge acceleration "runs" such as 4.5 g for 15 seconds. It fell from 98% to 93%. Since the onset of this change was delayed by more than 10 seconds, it occurred too late to affect venous oxygen saturation significantly during the period of the greatest depression of the saturation. This was during the first 10 seconds of the 15 second runs. As will be shown, the most striking result has been the maintenance of cerebral venous oxygen saturation in spite of the fall in arterial pressure induced by the gravitation. No correction has been made for a possible slight fall in arterial oxygen saturation during the higher, prolonged accelerations. However, the error resulting from this omission would only depress the apparent venous oxygen saturation and give an impression of failure of circulatory sufficiency.

During both arterial and venous oximetry, the blood accumulated in the mechanical syringe constituted an integrated sample giving the mean value of the saturation throughout the sampling period. There was a seven to eight second delay before the oximeter revealed changes in the oxygen content. This lag was due to the time taken for passage of the blood from the brain capillaries to the needle (three to four seconds) and thence to the cuvette (four seconds).

Light signals in the peripheral and central fields of vision were employed to assess the visual impairment during the runs. The responses were recorded photographically, together with the other variables under study.

RESULTS

1. *Cerebral venous oxygen content in relation to mean arterial pressures*

In Figure 1 mean arterial blood pressure at head level is compared with mean venous oxygen saturation. The pressure fell in direct proportion to the acceleration, attaining 30 mm. Hg at approximately 4.0 g.[3]

The mean cerebral venous oxygen saturation stays materially unchanged in contrast with the mean arterial pressure. In fact, two of the three series actually show a slight rise in the 2 to 3 g range. Some fall occurs at higher accelerations but never to the critical values that would be expected in view of the fall in arterial pressure. Fast records of the behavior of the venous oxygen content during the acceleration show the same picture. Figure 2 is a typical example of three records taken of two of the subjects, showing changes in oxygen saturation in the jugular vein as recorded by direct

[3] In extensive studies of arterial pressure at eye level during 15 second accelerations of abrupt onset, Lambert and Wood (3) have obtained similar results. The main difference is the acceleration at which they observed visual symptoms. They found that blackout normally occurs at 4 to 5 g and their data indicate that the lowest systolic pressure inducing a blackout during exposure was often zero, and generally below 20 mm. Hg. In the present study the mean arterial pressure, as measured throughout the run, was still approximately 30 mm. Hg at 4.5 g. Higher accelerations were needed to produce visual symptoms in these experiments because in order to give time for sampling, the centrifuge was accelerated gradually and the run lasted from one to two minutes. This technique elicits a full vasomotor response and consequently the arterial pressure was higher than in runs used by Wood and his associates (4).

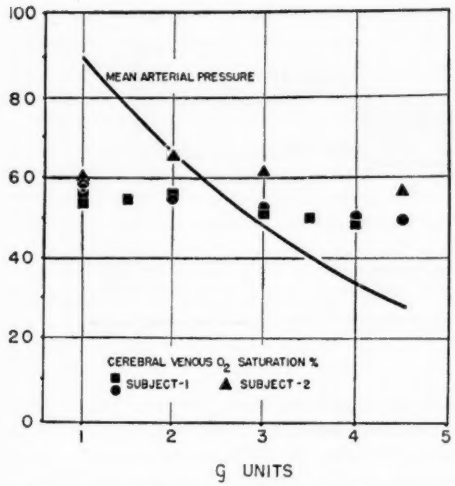

FIG. 1. MEAN ARTERIAL PRESSURE AT HEAD LEVEL PLOTTED AGAINST ACCELERATION

Ordinate: pressure in mm. Hg and venous oxygen saturation per cent. Abscissa; g units. Cerebral venous oxygen saturation remains essentially constant in spite of the fall in arterial pressure during the prolonged one to two minute accelerations.

oximetry. Events in the curve are delayed for seven to eight seconds by the time constants of the oximeter. After the initial increase from 50% to 55%, the saturation falls to 42% at the time when the arterial pressure is lowest. The vasopressor response is associated with a rise in venous saturation which follows the behavior of the blood pressure after the acceleration ends. However, these changes in oxygen saturation are small in comparison with the great fluctuations in arterial pressure at head level.

Since the arterio-venous oxygen difference is a measure of the sufficiency of the cerebral blood supply, some compensatory effect must be responsible for its remarkable constancy in face of the changes in arterial pressure. This effect could either be vasodilatation reducing cerebral vascular resistance or a fall in venous pressure increasing the force driving the blood through the capillary bed.

2. Pressure Measurements in the Jugular Bulb

It seemed possible that as a result of the acceleration, a negative pressure might develop in the intracranial veins which continuously sustained cerebral blood flow and, therefore, venous oxygen saturation, by partially compensating for the marked fall in cerebral arterial pressure. In addition to animal studies using the saggittal sinus as a pressure source (*7*), repeated measurements

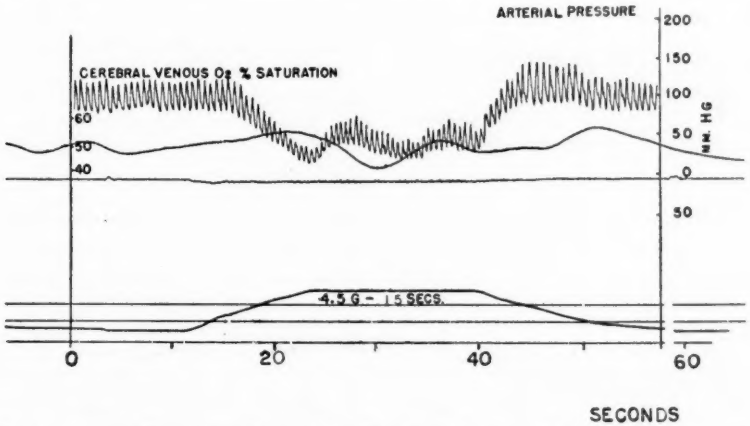

FIG. 2. ARTERIAL PRESSURE IN SUBJECT 3 AT HEAD LEVEL DURING A BRIEF 4.5 G ACCELERATION, LASTING 15 SECONDS

Abscissa; time in seconds. Ordinate; on left: cerebral venous oxygen saturation in volumes per cent; on the right: arterial pressure in mm. Hg. The constants of the oximeter cause a seven second delay in the response curve.

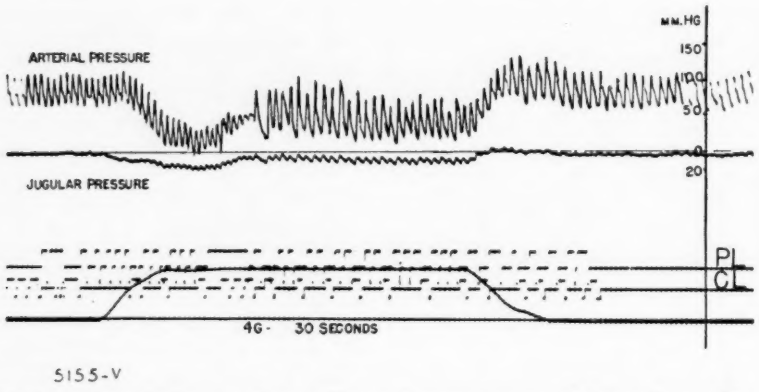

FIG. 3. CEREBRAL ARTERIAL AND JUGULAR VENOUS PRESSURES DURING A 30 SECOND RUN AT 4.0 G
Peripheral lights (PL) and center lights (CL) responses respectively. Note the failure to respond to the peripheral lights for a few seconds after the deepest fall in arterial pressure.

were taken at the superior jugular bulb using three subjects submitted to both brief and prolonged accelerations. In each case the pressures remained subzero throughout the acceleration. Brief accelerations of abrupt onset gave the most marked pressure falls and records showing the highest and lowest values obtained with such runs are presented. In the third subject the results were intermediate but of an identical pattern. In one run the arterial and jugular pressures were recorded during a 30 second acceleration (Figure 3). Seven seconds after the initial fall in arterial pressure, the vasopressor response took effect and the arterial pressure recovered to some extent. The first and third interrupted lines below the venous pressure curve, respectively, register response to peripheral and central lights (24). This case lost his peripheral lights for 3.5 seconds at a mean arterial pressure

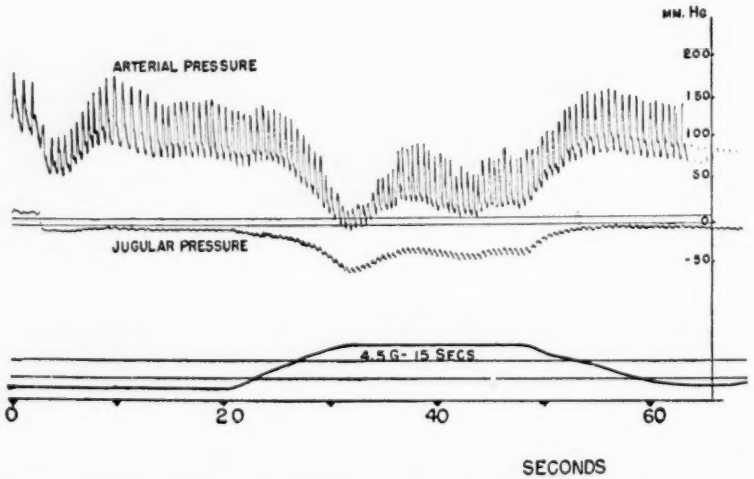

FIG. 4. CEREBRAL ARTERIAL AND JUGULAR VENOUS PRESSURES DURING A 4.5 G, 15 SECOND RUN, SHOWING THE CONTINUED RETENTION OF A HIGH ARTERIO-VENOUS PRESSURE DIFFERENTIAL DUE TO A MARKED FALL IN JUGULAR PRESSURE DURING THE ACCELERATION

of 10 to 15 mm. Hg, then during the vasopressor response, the mean arterial pressure rose to 50 mm. Hg and stabilized at 35 mm. Hg. The jugular pressure fell to 20 mm. Hg negative pressure at the time of the greatest fall in arterial pressure. Later, as the arterial pressure rose, the jugular pressure became less negative but still remained 8 mm. Hg less than ambient. At critical levels of cerebral arterial pressure, this addition could represent the difference between unconsciousness and adequate responses (see Table I).

In another case the jugular suction effect was much greater (Figure 4), and the fall was momentarily approximately 60 mm. Hg. Although the mean arterial pressure was less than 10 mm. Hg for three to four seconds, and less than 30 mm. Hg for more than six seconds, only mild visual dimming occurred. This may be because the venous pressure followed the arterial pressure changes and the cerebral arterio-venous pressure differential was never less than 50 mm. Hg. It was noted that whenever the onset of the acceleration was gradual, the magnitude of the jugular pressure fall was far less. A possible explanation for this will be advanced in the discussion.

DISCUSSION

These experiments demonstrate that there is some mechanism which compensates for the fall in cerebral arterial pressure induced by gravitational stress. Scheinberg and Stead (18) have also observed that when tilting patients into the erect posture, the fall in arterial pressure induced by gravity stress was of greater extent than that of the cerebral blood flow. They too, point out that the apparent fall in cerebral vascular resistance could either be due to a mechanical pressure decrease on the venous side of the capillary bed, or to active cerebral vasodilation. An additional effect could be a passive dilation of the brain vessels brought about by a fall in venous pressure. It is possible that all of these mechanisms may be in operation at the same time.

When the stress is imposed rapidly, the ensuing subatmospheric pressures which have been demonstrated in the jugular bulb of men and the saggittal sinus of cats and dogs (25, 7) are produced almost instantaneously. Any active dilation mediated through the nervous system would require several seconds. Not only does the subatmospheric pressure effect develop rapidly, but also it is sufficiently intense to account for the continued blood flow and high venous oxygen saturation, in a large part. However, at no time is it large enough to completely balance the fall in arterial pressure. Even in the case shown in Figure 4, the arterio-venous pressure differential sinks approximately to one half of its normal value during the run. The fact that cerebral vascular sufficiency, as indicated by the cerebral arterio-venous oxygen difference, is hardly affected by such a marked fall in the driving pressure makes it probable that the flow is sustained by a decrease in cerebral vascular resistance which assists the direct pull of the venous pressure fall.

There is a purely mechanical means by which such a fall in resistance may be brought about by a pressure decrease on the venous side of the capillary bed. A drop in pressure in the venous plexus about the spinal cord during acceleration in the upright seated posture would lead to collapse of these veins by drainage into the lower abdominal system. This space left by them would be filled by cerebro-spinal fluid draining from the cranial cavity. Thus a passive distension of the cerebral vessels might occur to fill the gap left by the cerebro-spinal fluid. During the distension of the veins a small amount of blood will be aspirated from the arterial tree. It was this brief excess of blood flow that Ranke (26) and others (25) believed might play some part in preventing loss of consciousness during short-lasting accelerations. For it has long been known that venous and cerebro-spinal fluid pressure in the head becomes negative in man when he assumes the erect posture (27). Ranke (26), Jasper and Cypriani (28), and Rushmer, Beckman and Lee (25) also recognized that in animals these pressures become strongly subatmospheric during positive acceleration. It has not been generally realized that significant deep channels, which can remain patent in spite of subatmospheric pressures, are available for the return of blood from the brain. Nor, as a matter of fact, has the partition into the various available channels of the blood returning during acceleration yet been analyzed.

It is now appreciated that the internal and external vertebral venous plexes (29) are extensive and the internal system is protected from collapse during acceleration by the subatmospheric cere-

bro-spinal fluid pressure (25). Therefore, an uninterrupted flow can continue down these vessels. The fact that edema of the face has been reported following blocking of the superior vena cava suggests that in man the capacity of the subsidiary channels is limited and that extra pressure is needed to force the full cerebral blood flow through them (30). Thus any rise in arterial pressure, or active or passive vasodilatation tending to increase blood flow during acceleration would be partially offset by a rise in pressure in the jugular bulb. When the pressure in this region becomes atmospheric, then it may be assumed that the jugular veins become available and the resistance to flow would fall accordingly. Such a mechanism would explain the simultaneous fluctuations of arterial and venous pressures observed in these records. For, as arterial pressure fell and flow decreased, the pressure in the jugular bulb at the entrance to the cervical venous system would fall and the venous pull would increase correspondingly. It also explains why when the gravitational stress is imposed slowly and there is adequate time for a vasopressor response, the fall in jugular pressure is far less pronounced than during the first few seconds after an abrupt acceleration when the fall in cerebral arterial pressure is most marked.

The increased pulsation in the jugular tracing during the acceleration would suggest a vasodilatation (Figures 3 and 4). These waves do not originate from the auricle and are not transmitted up to the jugular vein, for they are not eliminated by manual occlusion of the vein during acceleration. They might be due to the direct transmission of the arterial pulse through passively dilated cerebral vessels. Alternatively, the venous pulsations may be due to direct transmission of volume changes from arteries to veins across the tissues of the brain. The pulsations would become more intense when the cerebral arterial pressure decreased during the acceleration because the arteries are not then so completely distended and would, therefore, fluctuate in volume more at any given pulse pressure.

There is some evidence of active cerebral vasodilatation during acceleration. Thus it was found that the cerebral venous oxygen saturation in anesthetized animals exposed to positive acceleration remained elevated for several minutes after the run in spite of normal arterial pressures (31). It is of interest in this connection that of the two 1 g controls in the series identified by the squares in Figure 1, the higher value of 57.2% was for blood taken immediately after a series of prolonged runs. However, data such as these can, at best, only be regarded as suggestive. Fortunately, Kety and his associates (14) have recently shown that when in hypertensives, the blood pressure is reduced by spinal sympathetic block, there is a partial relaxation of the cerebral vessels which tends to counteract the decrease in cerebral flow which would otherwise occur. This reflex relaxation does not compensate as fully for the fall in blood pressure as do the various mechanical and nervous factors at play during gravitational stress. This can be seen by comparing Figure 1 in their article with Figure 1 in the present article. A reduction of the arterial pressure to one half its normal value has less influence upon the venous oxygen saturation in the cases undergoing acceleration. When contrasted with the results of gravity induced cerebral hypotension, the data of Kety's group throw light upon the relative roles played by mechanical factors and by active cerebral vascular relaxation in the determination of cerebral blood flow, always provided that such relaxation also occurs in normal persons. Also the observation that relaxation of the cerebral vessels appeared to be independent of the degree of hypertension does suggest that a similar relaxation may occur in normotensive individuals suffering a sudden fall in blood pressure.

If the arterial pressure falls below the value necessary to force the blood to head level and hold open the carotid vessels and the cerebral vascular bed, then unconsciousness will occur no matter how perfect the compensation provided by suction and other factors. Animal experiments (31) have suggested that at high accelerations, when the pressure falls too low to fill the carotid arteries up to the level of the base of the brain, they collapse and flow through the carotid system ceases abruptly. Although in the dog the remaining flow through the pressure protected vertebral and spinal vessels may be adequate to maintain consciousness (5, 32), in man this is not so. Consequently, in contrast with the dog (31), in man consciousness is lost when arterial pressures at head level become subzero.

The fact that slightly higher blood pressures are required to maintain vision when protection against the effects of acceleration is afforded by pneumatic anti-G suits (33) may be due to the increase in central venous pressure and in cerebral-spinal fluid pressure produced by the inflation of such suits.

Ernstene and Blumgart (34) have suggested that one of the reasons for the orthopnoea of congestive heart failure might be an improvement in the cerebral circulation that resulted from the lowering of the cerebral venous pressure in the erect position. It is possible that some significance should be attached to this factor (35, 36). Certainly, the evidence just presented has shown that when a subject is placed in the erect position in a high gravitational field, the cerebral vascular sufficiency may remain undisturbed in spite of a great fall in arterial pressure. It is not yet clear whether this occurs solely as a direct result of the decrease in pressure on the venous side of the cerebral capillaries or whether passive vasodilatation also occurs due to an expansion of the cerebral vascular bed, but it is probable that whenever the acceleration persists for more than a few seconds, a combination of these effects develops, together with some active cerebral vasodilatation.

SUMMARY

1. Consciousness is usually lost when the mean cerebral blood pressure falls to 25 mm. Hg, but during exposure to high accelerations such as those producing aviator's blackout, alertness may be retained in spite of such mean pressures.

2. Arterial and cerebral venous oxygen saturation and arterial pressure at head level and cerebral venous pressures were measured in three subjects during acceleration. The venous saturation remained almost unchanged in spite of great falls in cerebral arterial pressure. This suggested that some factor was maintaining cerebral blood flow.

3. Pressures ranging from 20 to 60 mm. Hg below ambient were found in the jugular bulb. This effect could maintain consciousness by sustaining the arterio-venous pressure differential, or by producing a combination of such an effect with passive cerebral vasodilatation.

4. There is also evidence that active cerebral vasodilatation may occur during prolonged exposure to gravitational stress in the erect posture.

ACKNOWLEDGMENTS

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CORRELATION OF THE 24-HOUR RADIOIODINE UPTAKE OF THE HUMAN THYROID GLAND WITH THE SIX- AND EIGHT-HOUR UPTAKES AND THE "ACCUMULATION GRADIENT"

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Techniques for the determination of thyroid function have become increasingly refined since the demonstration of the relation of basal oxygen consumption to thyroid activity 50 years ago. Newer methods include the determination of serum protein-bound iodine and various procedures using radioactive iodine. The latter include direct measurements of the accumulation in the thyroid gland of an administered dose of radioactive iodine (1), studies of urinary excretion (2), and observations of the rate of formation of serum protein-bound radioiodine (3). All observers agree that there is an elevated rate of accumulation of radioactivity in the thyroid gland in hyperthyroid, a depressed rate in myxedematous, and an intermediate rate in euthyroid subjects. This is true whether the uptake is measured directly over the gland or indirectly by excretion curves.

There is no standardized procedure at present among various institutions using radioactive iodine, but the general preference seems to be for techniques measuring the accumulation of radioiodine directly over the thyroid rather than by excretion curves. Probably the measurement of radioactivity in the thyroid gland 24 hours following the administration of I^{131} (4) is most commonly used.

Astwood and Stanley (1) found that if the radioactivity in the thyroid is measured at frequent intervals following the administration of a tracer dose of I^{131}, the concentration in the thyroid is a linear function of the square root of time. The slope of the line expressing this linear relationship is constant for the first eight to 12 hours in euthyroid subjects and has been called the "accumulation gradient." By means of this technique it is possible to obtain rapidly an evaluation of thyroid function and it is quite valuable in determining the effect of various experimental procedures on the function of the thyroid gland. For example, if an antithyroid compound is administered following the initial determination of the gradient, the relative activity of the drug can be judged by the extent to which further accumulation of I^{131} is inhibited (5). Conversely, the effectiveness of an injection of thyrotrophin can be judged by the increase which it produces in the gradient (6).

Two important objections have been raised to using the accumulation gradient as an index of thyroid function, however. In the first place, no physiologic significance has yet been discovered for the finding that the early course of radioiodine accumulation follows a parabolic curve. Secondly, Astwood and Stanley described their technique during the early development of accurate counting devices and were limited to a small, shielded, Geiger-Mueller counter which was placed in direct contact with the skin overlying the thyroid. Consequently absolute determinations of radioactivity in the thyroid could not be made and the values were recorded only as "counts per second per 100 microcuries of administered I^{131}." This made it impractical to utilize the observations of these investigators in other laboratories since the counting device would have to be duplicated exactly in order to obtain comparable data. Data expressing the radioactivity in the gland as a fraction of the administered dose can invalidate the second objection.

The present investigation was undertaken in an attempt to correlate the 24-hour uptake with other indices of thyroid function involving the use of I^{131}. All measurements were made with an adequately shielded Geiger-Mueller counter at a distance of 55 cm. from the neck. The absolute

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values obtained can thus be compared with those of other investigators. The accumulation gradient, 6-hour, 8-hour, and 24-hour uptakes were determined in the same individual and the other three functions then correlated separately with the 24-hour uptake.

MATERIALS AND METHODS

All subjects were healthy, young, clinically euthyroid volunteers, predominantly female technicians. Separate determinations of all four indices were made in 75 instances using 38 subjects. Twenty-eight subjects were employed more than once, none more than three times. Those studied more than once were given oral desiccated thyroid following the initial determination.

A shielded Sylvania gamma counter (GG-306) was so arranged that only the region directly surrounding the thyroid would be counted when the apparatus was in position. A Tracerlab Autoscaler was used to total the counts. A device was employed which aligned the aperture of the shielding over the center of the thyroid gland and which could also be arranged to support a flask containing I^{131} at the same distance, when required. All counting was done at a distance of 55 cm. from the end of the Geiger-Mueller tube to the skin over the thyroid gland. Using the tube end-on the effective center of the counting volume is 6 cm. from the near end of the tube. The apparatus is to be described in detail in a future communication (7).

Approximately 50 microcuries of carrier-free I^{131} were pipetted into either a small 50 cc. glass beaker or Erlenmeyer flask, diluted with 15–20 cc. of tap water, and the absolute radioactivity determined at a distance of 55 cm. from the gamma counter to the near surface of the vessel. The subject was thereupon given the solution to drink. This was followed by a rinsing of another 15–20 cc. of tap water to facilitate removal of the radioactivity from the flask, mouth, and pharynx. A single rinsing was sufficient to remove 99 per cent of the radioactivity from the flask.

The thyroid region of the subject was then counted at approximately hourly intervals during the day. Correction was made for background radiation and extrathyroidal I^{131} by subtracting counts made over the lower thigh of the subject. The thigh count is somewhat less than the count contributed by the non-thyroidal tissues of the neck and this is therefore an undercorrection. The error thus introduced is not large, however, and is not significant except during the first hour or two after the tracer dose is given (8).

Since the counting was done at a distance of 61 cm. from the skin to the effective counting center of the Geiger-Mueller tube, small variations in the depth of the center of the thyroid gland from the skin would have little effect on the number of impulses reaching the tube. It seemed unnecessary, therefore, to make any correction for the estimated center of the thyroid gland since the radioactivity in the flask was also measured with its near surface 61 cm. from the effective counting center of the tube. The calculated radioactivity in the gland was increased somewhat by back scatter from the cervical region, but this would seem to be far less (9) than that described by Myant, Honour, and Pochin (10).

TABLE 1

x	Formula	S. D.	S. E.
Accumulation gradient	$y = 31.2x - 3.26x^2$	$\pm 0.141y$	$\pm 0.0164y$
6-hour uptake	$y = 1.67x$	$\pm 0.129y$	$\pm 0.0153y$
8-hour uptake	$y = 1.43x$	$\pm 0.134y$	$\pm 0.0160y$

The accumulation gradient was determined by plotting graphically the *per cent* of administered radioactivity present in the thyroid against the square root of the time in minutes following administration. The slope of this line was the numerical value of the accumulation gradient. The various gradients, 6-hour and 8-hour uptakes were then plotted, respectively, against the corresponding 24-hour uptakes.

RESULTS

A close correlation was found to exist between the 24-hour uptake and all other functions. In the case of the 6-hour and 8-hour uptakes, simple straight line formulae were found to fit the data quite well. Since the spread was greater with the higher uptakes, the standard deviation and standard error were calculated as a fraction of the calculated quantity rather than as absolute values. A straight line could not be fitted to the data correlating the accumulation gradient and the 24-hour uptake, but a parabolic curve having the formula $y = 31.2x - 3.26x^2$ was found to fit quite pre-

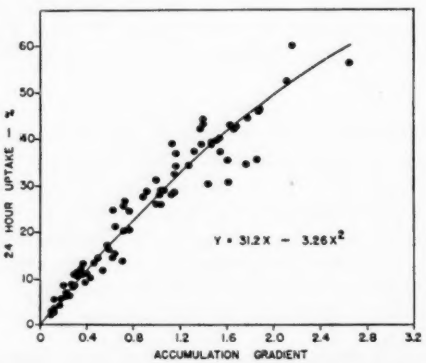

FIG. 1. RELATION OF ACCUMULATION GRADIENT TO 24-HOUR UPTAKE OF I^{131} BY THE THYROID

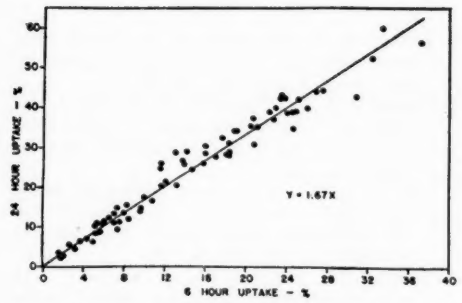

FIG. 2. RELATION OF 6-HOUR UPTAKE TO 24-HOUR UPTAKE OF I^{131} BY THE THYROID

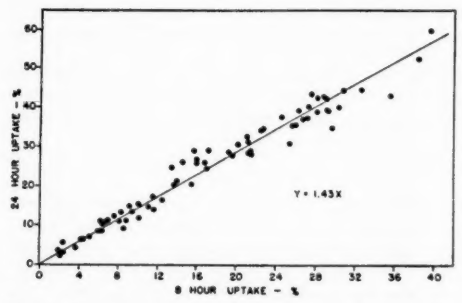

FIG. 3. RELATION OF 8-HOUR UPTAKE TO 24-HOUR UPTAKE OF I^{131} BY THE THYROID

cisely. The formulae for the various correlations with the 24-hour uptake are listed in Table I. In each instance y represents the 24-hour uptake plotted on the ordinate and x represents the other functions, respectively, plotted on the abscissa. The formula for the accumulation gradient was calculated by the method of least squares. (Figures 1–3).

DISCUSSION

The use of the data in this investigation makes possible the translation of the accumulation gradient, 6-hour, 8-hour, or 24-hour uptake in a euthyroid subject into comparable terms. Thus if a subject has an accumulation gradient of 1.6, using the formula $y = 31.2x - 3.26x^2$, the 24-hour uptake can be calculated to be 41.7 ± 6.9 per cent. The standard deviation of ± 6.9 is obtained by taking 14.1 per cent of y, in this instance 41.7 per cent.

The 24-hour uptake can thus be transformed into the 6-hour or 8-hour uptake by dividing by 1.67 or 1.43, respectively.

Such translations are chiefly of value in observations of normal or hypothyroid subjects. It is unlikely that the values obtained in thyrotoxic subjects would exhibit the same degree of correlation since the rate of uptake is often so rapid that the parabolic curve is followed for only a short time, making the gradient inaccurate. Also, some hyperthyroid patients exhibit a significant loss of activity from the gland following the first two to three hours after administration of I^{131}. This is presumably because a hyperactive gland contains only a small store of thyroxin which therefore is of unusually high specific activity. The higher rate of discharge in thyrotoxicosis serves further to accentuate this loss of radioactivity from the gland. In euthyroid subjects this does not occur since the organically bound I^{131} serves to label a large "pool" of thyroxin and thyroglobulin in the colloid from which secretion takes place quite slowly. Consequently accurate predictions of the 24-hour counts from the accumulation gradients of thyrotoxic individuals are probably not possible.

The formula expressing the relation between the 24-hour uptake and the accumulation gradient is incorrect for the higher rates of accumulation seen in thyrotoxicosis. The parabola formed by this formula reaches a maximum at a gradient of 4.8. The corresponding 24-hour uptake is 75.8 per cent, obviously too low since many patients with severe hyperthyroidism have uptakes above 90 per cent.

The relatively large number of very low uptakes and gradients reported here for euthyroid subjects are due to the fact that these subjects had been given exogenous thyroid hormone as part of another study. It has been found that such therapy will produce a diminution in thyroid function, as measured by studies with I^{131}, to levels seen in myxedema (11). In every such instance, a previous uptake before thyroid medication had been instituted had shown a normally functioning gland. Although the activity of these thyroids was suppressed, no change was produced in the correlation of the various functions studied. It was of advantage to study subjects whose thyroid glands

were suppressed to various degrees by exogenous hormone since in this manner many points could be calculated between very low and high uptakes, otherwise a difficult task.

SUMMARY

The correlation of the accumulation gradient, 6-hour, and 8-hour uptake with the 24-hour uptake of radioactive iodine by the thyroid gland has been investigated in euthyroid human subjects. A close correlation has been found to exist between all four functions. If y is taken to represent the 24-hour uptake and x the various other functions, relatively precise formulae can be constructed by means of which the 24-hour uptake can be calculated from any of the other functions and vice versa. Thus, $y = 31.2x - 3.26x^2 \pm 0.141y$ is the formula for the accumulation gradient, $y = 1.67x \pm 0.129y$ is the formula for the 6-hour uptake, and $y = 1.43x \pm 0.134y$ is the formula for the 8-hour uptake. These correlations hold even when the thyroid gland is suppressed by exogenous thyroid hormone. The data presented here may be of value in future investigations of thyroid physiology in euthyroid subjects. It is suggested that far less correlation would be expected in thyrotoxic subjects.

ACKNOWLEDGMENT

I am indebted to Dr. E. B. Astwood and Dr. J. F. Ross for helpful advice and suggestions in the preparation of this manuscript.

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THE EFFECT OF THE UPRIGHT POSTURE UPON HEPATIC BLOOD FLOW IN NORMOTENSIVE AND HYPERTENSIVE SUBJECTS [1]

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INTRODUCTION

The upright posture imposes a strain upon the cardiovascular system, chiefly as a result of its hydrostatic effects in the lower half of the body which, if unopposed, tend to produce hypotension in the upper half. These hydrostatic effects normally are counteracted by autonomic vasomotor adjustments, particularly in the splanchnic region. For example, the upright posture in man usually causes a reduction of renal blood flow (2). However, as yet no direct evidence has been reported concerning its effects upon the largest remaining portion of the splanchnic circulation, namely that draining through the liver. The present communication reports measurements of "hepatic blood flow" obtained with the bromsulfalein (BSP) extraction method (3) in normotensive and hypertensive subjects studied in the supine, in the upright, and again in the supine position.

METHODS

The estimated hepatic blood flow (EHBF) was measured by the intravenous catheterization method of Bradley and associates (3). The continuous infusion of BSP was most easily maintained at a constant rate in both the horizontal and upright positions by suspending the infusion flask from a pole attached to the tilt table at such an angle that the flask remained at the same distance above the level of the subclavian vein when the table was horizontal or tilted up at 75°.

The subjects were hospital patients selected for study either because they had essentially normal cardiovascular systems or had arterial hypertension without evidence of cardiac, renal or hepatic insufficiency. All were studied in the basal state, at first supine, then tilted (passively) into the upright (75°) position for periods of 10 to 25 minutes (unless weakness and hypotension supervened), and finally, supine again. They were requested to remain as relaxed and motionless as possible throughout the test. Arterial pressure and pulse rate were measured either by the usual clinical methods or (in Cam, Aye and Dun) with a Hamilton manometer (4) at short intervals during each experiment.

Spot roentgenograms of the hepatic area were taken in each patient before, during, and after the upright tilt to demonstrate that the catheter remained properly placed in the same position near the center of the right lobe of the liver. To avoid possible errors due to mechanical and hydrostatic disturbances incident to changing the position of the subject, no result was computed on blood samples obtained within three minutes after a tilt, except for a few determinations made on blood samples that were taken immediately after a quick return to the horizontal position necessitated by faintness of the patient. As noted in Table I, such determinations have been related to the period just before rather than after this change of position because there appeared to be a time lag of one to two minutes before there was evidence of any acute change in hepatic blood flow as reflected in altered concentrations of BSP in the blood samples. After this time lag, however, considerable changes in serum BSP concentration could take place with great rapidity, especially when the subject had been tilted from the supine to the upright position. About four minutes after a tilt the trend of the BSP levels became more steady and the results after this time were accepted as more reliable measurements of an equilibrium state than those obtained earlier after a change in position. Furthermore, some subjects tolerated the upright position easily (top of Table IA and IB) and the results in them during upright tilting were regarded as more reliable than in the less stable subjects, some of whom fainted (bottom of Table IA and IB, and Figure 3).[4]

An effort was always made to draw the last pair of blood samples in each period as late as possible before a change in tilt table position. All estimates of rate of blood flow were calculated for the actual times of sampling and on the observed rather than the interpolated

[1] Presented May 5, 1947 at the Thirty-Ninth Annual Meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey (1).

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[4] Only those data obtained after four minutes in a given position, and without fainting, are included in the statistical analysis in Table II.

TABLE I

Effects of the upright posture upon estimated hepatic blood flow (EHBF), mean (half systolic plus diastolic) arterial pressure, calculated hepatic-portal resistance (HPR) and pulse rate in normotensive (A) and hypertensive (B) subjects, arranged in order of their relative adaptability (freedom from symptoms and signs of poor postural response) to the upright posture

Patient Age Sex Surface area (sq. m.)	Horizontal position, Control period				Upright (75°) position					Horizontal position, Recovery period				
	EHBF (cc./min./1.73 sq. m.)	Mean arterial pressure (mm. Hg)	HPR (mm. Hg/cc./sec./1.73 sq. m.)	Pulse rate (Beats/min.)	Minutes after tilt-up	EHBF (cc./min./1.73 sq. m.)	Mean arterial pressure (mm. Hg)	HPR (mm. Hg/cc./sec./1.73 sq. m.)	Pulse rate (Beats/min.)	Minutes after tilt-back	EHBF (cc./min./1.73 sq. m.)	Mean arterial pressure (mm. Hg)	HPR (mm. Hg/cc./sec./1.73 sq. m.)	Pulse rate (Beats/min.)
							(A)							
Ema														
39	1,924	98	3.1	68	4½	1,143	97	5.1	104	8½	1,858	100	3.2	72
M	1,716	103	3.6	76	11	1,312	90	4.1	98	15½	1,713	103	3.6	74
1.70														
Ols														
32	2,600	99	2.3	85	5	1,339	106	4.8	96	10	1,210	92	4.6	72
M	—	—	—	—	13	1,375	102	4.5	90	20	1,155	91	4.7	84
1.89	—	—	—	—	21	1,466	100	4.1	90	—	—	—	—	—
Sil														
25	1,762	101	3.4	100	4	1,319	99	4.5	120	5	1,381	95	4.1	102
M	1,603	100	3.7	99	7	1,505	99	3.9	132	11½	1,900	95	3.0	96
1.98	—	—	—	—	13	1,203	101	5.0	132	18	1,807	101	3.4	96
					18	1,381	104	4.5	138					
Tef														
29	1,500	106	4.2	80	5½	762	99	7.8	132	3	1,115	112	6.0	96
M	1,411	105	4.5	80	13½	680	96	8.5	132	7	1,247	112	5.4	90
2.08	—	—	—	—	—	—	—	—	—	14½	1,094	105	5.8	80
Gio														
24	1,107	98	5.3	86	6	50*	78	?	108	3½	944	97	6.2	92
M	2,164	95	2.6	88	12½	796	91	6.9	108	19½	1,535	98	3.8	92
1.78	—	—	—	—	21	978	89	5.5	104	31	1,034	99	5.8	84
Car														
24	1,101	103	5.6	88	5	818†	88	6.5	114	3½	142*	102	?	102
M	1,082	101	5.6	87	—	—	—	—	—	8½	874	98	6.7	82
1.76	—	—	—	—	—	—	—	—	—	15½	912	100	6.6	84
Fau														
28	920	98	6.4	68	6½	703*‡	75	?	+?	6½	962	97	6.1	58
M	724	95	7.9	68	—	—	—	—	—	—	—	—	—	—
2.07														
McC														
22	1,126	87	4.6	72	5½	1,174*‡	96	4.9	114	7½	644	89	8.3	63
M	1,040	84	4.8	72	8	505	?	?	?	13½	864	90	6.3	66
1.76														

TABLE I—*Continued*

Patient Age Sex Surface area (*sq. m.*)	Horizontal position, Control period				Upright (75°) position					Horizontal position, Recovery period				
	EHBF (*cc./min./ 1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*)	Pulse rate (*Beats/min.*)	Minutes after tilt-up	EHBF (*cc./min./ 1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*)	Pulse rate (*Beats/min.*)	Minutes after tilt-back	EHBF (*cc./min./ 1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*)	Pulse rate (*Beats/min.*)
							(B)							
McC														
43	1,624	183	6.8	68	3	1,450	179	7.4	80	3½	1,380	193	8.4	68
M	1,503	180	7.2	62	8	1,211	180	8.9	84	13½	1,591	188	7.1	64
1.75	—	—	—	—	18½	1,461	185	7.6	86	23½	1,844	190	6.2	65
She														
51	961	131	8.2	64	6	702	141	12.0	84	12	949	134	8.5	64
M	1,298	142	6.6	67	16	979	137	8.4	87	20	892	130	8.7	64
2.02	—	—	—	—	24½	760	141	11.0	88	—	—	—	—	—
Cam														
51	1,118	167	9.0	70	6	1,072	175	9.8	84	5	1,036	156	9.0	60
M	—	—	—	—	11	1,059	171	9.7	88	10½	900	—	—	—
1.71	—	—	—	—	16	998	170	10.0	83	—	—	—	—	—
Val														
37	1,217	162	8.0	76	3	566	174	18.0	90	3	768	152	12.0	72
M	1,440	152	6.3	70	7½	825	165	12.0	112	10½	1,224	170	8.3	84
2.05	—	—	—	—	14½	969	163	10.0	108	17	1,141	160	8.4	75
Max														
43	1,466	133	5.4	56	7	876	127	8.7	72	4½	2,650§	136	3.1	66
M	1,590	130	4.9	60	12	713	128	11.0	84	14	3,164§	143	2.7	60
2.21	—	—	—	—	20½	694	131	11.0	?	24	2,050	133	3.9	60
Ric														
33	1,077	174	9.7	68	6	859	184	13.0	115	7	1,575	—	—	—
M	1,312	176	8.0	68	8	586	161	16.0	120	18	1,347	—	—	—
1.91														
Dea														
54	1,462	159	6.5	58	6	1,154	164	8.5	66	10½	1,363	168	7.4	60
M	1,227	177	8.6	64	10	1,041	179	10.0	69	22½	1,444	168	7.0	56
1.82														

TABLE I—*Continued*

Patient Age Sex Surface area (*sq. m.*)	Horizontal position, Control period				Upright (75°) position					Horizontal position, Recovery period				
	EHBF (*cc./min./ 1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*)	Pulse rate (*Beats/min.*)	Minutes after tilt-up	EHBF (*cc./min./ 1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*)	Pulse rate (*Beats/min.*)	Minutes after tilt-back	EHBF (*cc./min./ 1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/ cc./sec./ 1.73 sq. m.*)	Pulse rate (*Beats/min.*)
							(B)—*Continued*							
Gol														
31	1,095	179	9.8	84	6	840	158	11.0	90	4½	802	174	13.0	72
M	980	177	11.0	78	—	—	—	—	—	9	1,123	175	9.4	72
1.81										14½	1,215	176	8.7	75
										19½	1,168	176	9.0	75
Pau														
36	1,964	124	3.8	66	5½	1,125	123	6.6	90	8	1,895	119	3.8	60
M	—	—	—	—	—	—	—	—	—	13	1,505	114	4.5	64
1.84	—	—	—	—	—	—	—	—	—	23	1,750	126	4.3	75
Aye														
51	1,409	—	—	—	5½	863†	100	7.0	62	6	772	120	9.3	58
M	1,408	164	7.0	72	—	—	—	—	—	11½	928	125	8.1	56
1.86										21½	916	131	8.6	53
Dun														
30	1,067	115	6.5	84	3	658†	?	?	?	4	750	124	9.9	81
M										12½	953	104	6.5	90
1.82														
Col										3	752	139	11.0	72
28	1,039	174	10.0	90	4½	740‡	102	8.3	61	7	808	170	13.0	82
M	864	173	12.0	90	—	—	—	—	—	14½	1,478	172	7.0	84
1.72										24	894	170	11.0	90
										37	1,164	169	8.7	96

* This value was obtained during a rapid rise (more than 0.0006 mg./cc./min.) in the peripheral venous serum concentration of BSP. It is questionable, and is deleted from the statistical analysis (Table II).

† Patient faint, samples for EHBF taken, and immediately tilted back horizontal.

‡ Patient faint, tilted back horizontal, and samples for EHBF taken immediately.

§ Patient had severe headache and excitement.

values for serum concentration of BSP. Rate of change (ΔP) in the peripheral venous or (in Cam, Aye and Dun) arterial concentration of BSP was taken as the calculated average for the preceding period in each instance. This value never exceeded 0.0006 mg./cc./min., except in four instances when the subject had been hypotensive [5] (Table I). Blood volumes were calculated by the method of Gibson and Evans (5). All estimates of hepatic blood flow were corrected for standard surface area (1.73 sq.m.). Hepatic portal resistance (HPR) was calculated simply by dividing the "mean" (one-half systolic plus diastolic) arterial pressure by the EHBF per second.

A cold pressor test (6)—immersion of one hand in ice water for one minute—was applied to six (four normotensive and two hypertensive) subjects in the horizontal position after a period of recovery from the effects of the upright position. This was abandoned later because it was found to produce only equivocal effects upon EHBF.

RESULTS

A detailed presentation of data is given in Table I and a statistical summary in Table II. In both normotensive subjects (Table IA, Figure 1) and hypertensive patients (Table IB, Figure 2) the EHBF decreased following the tilt from the horizontal to the upright position. The decreases in EHBF were usually not associated with proportionate changes in mean arterial pressure, so that calculated hepatic-portal resistance (HPR) increased. Comparing only equilibrium values (Table II) the EHBF (cc./min.) decreased on the average from 1,713 to 1,070 in the normotensive group, from 1,357 to 960 in the hypertensive group, and from 1,499 to 1,004 in the combined groups. During the upright period, the average arterial pressure did not change, so that the HPR increased from 3.8 to 5.5 in the normotensive group, from 7.3 to 10.1 in the hypertensive group, and from 6.0 to 8.3 in the combined groups. These changes were significant statistically.

The changes in EHBF and HPR were not directly related to increases in pulse rate or to other signs and symptoms of poor circulatory adaptation to the upright posture (Table I). However, there was in general a correlation between the tendency of a subject to collapse and to have a low EHBF in the upright position. Thus, of the six subjects who fainted, four had an EHBF below 750 cc./min. during the upright period and another had it just after being tilted back horizontal. Although six of the ten subjects who had an upright EHBF below 750 cc./min. did not faint, three (Gio, She, Val) of them had the low flow only during the first six minutes after being tilted up, and on further standing had an EHBF above 750 cc./min.

[5] These data were omitted from the statistical analysis in Table II.

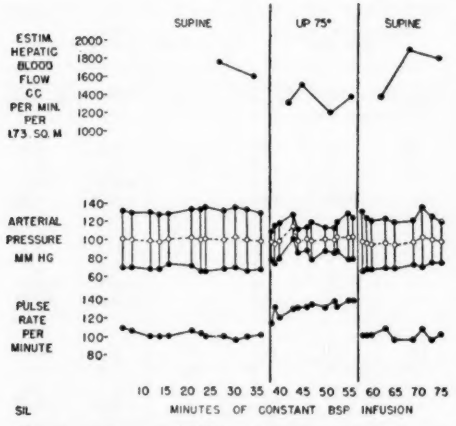

FIG. 1. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE IN A NORMOTENSIVE SUBJECT (SIL), IN THE HORIZONTAL, UPRIGHT, AND AGAIN IN THE HORIZONTAL POSITION

"Mean" arterial pressure (half systolic plus diastolic) is indicated by the open circles and interrupted line. At the first vertical line, the subject was tilted upright (75°) and at the second, horizontal again.

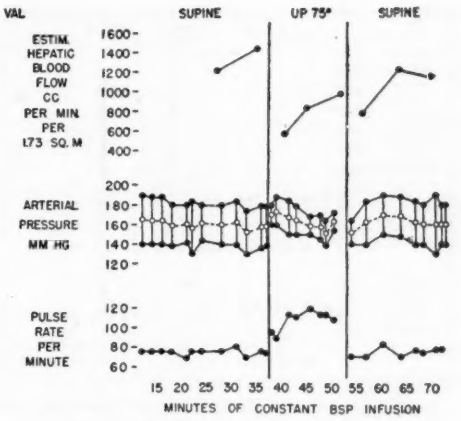

FIG. 2. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE IN A HYPERTENSIVE PATIENT (VAL)

Other notations as in Fig. 1.

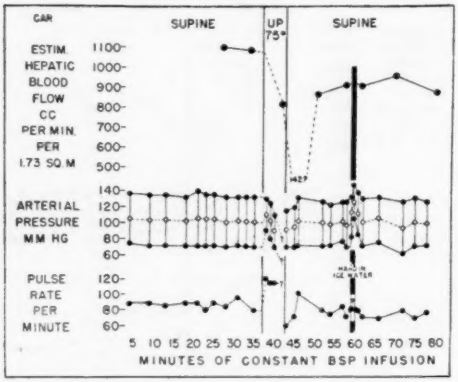

FIG. 3. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE IN A NORMOTENSIVE PATIENT (CAR)

At the heavy vertical line the left hand was immersed in ice water for one minute. Other notations as in Fig. 1.

Upon returning the subjects from the upright to the horizontal position the EHBF and HPR usually returned toward, to, or even beyond the control horizontal levels after varying periods of time. In some subjects the returns were prompt, in others they were delayed or incomplete, or occurred only after an initial overshoot beyond the previous horizontal values. In one hypertensive individual (Max) the post-tilt increases of EHBF and decreases of HPR were extreme and associated with a marked anxiety reaction that began with a severe headache.

Upon tilting the subjects back to the horizontal position the average values (Table II) changed as follows: EHBF rose from 1,070 to 1,312 in the normotensive group, from 960 to 1,417 in the hypertensive group, and from 1,004 to 1,372 in the combined groups, while the HPR decreased from 5.5 to 4.9, from 10.1 to 7.2, and from 8.3 to 6.2, respectively. Since the measurements during recovery showed greater variability than before tilting, the changes during this period, while significant for the combined and hypertensive groups, were not for the normotensive group considered separately.

Except in one patient (Sil), immersion of a

TABLE II

Mean values of estimated hepatic blood flow (EHBF), arterial pressure and hepatic portal resistance (HPR) in relation to posture, with a statistical analysis of the significance of the differences†*

	EHBF (*cc./min./1.73 sq. m.*)			Mean§ arterial pressure (*mm. Hg*)			HPR (*mm. Hg/cc./sec./1.73 sq. m.*)		
	Horizontal (Control)	Upright	Horizontal (Recovery)	Horizontal (Control)	Upright	Horizontal (Recovery)	Horizontal (Control)	Upright	Horizontal (Recovery)
Normotensives (n = 6)									
Mean	1,713	1,070	1,312	98	97	98	3.8	5.5	4.9
Standard Error of Mean	65	37	155	3	2	3	0.1	0.6	0.6
Mean Difference‡	—	−642	+242	—	−1	+1	—	+1.7	−0.6
Significance of Difference (P)	—	**<0.01**	0.12	—	0.75	0.81	—	**<0.03**	0.42
Hypertensives (n = 9)									
Mean	1,357	960	1,417	158	157	155	7.3	10.1	7.2
Standard Error of Mean	97	68	172	7	7	8	0.6	0.7	0.7
Mean Difference	—	−397	+428	—	−1	0	—	+2.8	−2.4
Significance of Difference (P)	—	**<0.01**	**<0.05**	—	0.78	0.97	—	**<0.01**	**<0.01**
Combined Groups (n = 15)									
Mean	1,499	1,004	1,372	134	133	131	6.0	8.3	6.2
Standard Error of Mean	107	61	116	9	9	9	0.6	0.8	0.6
Mean Difference	—	−495	+348	—	−1	0	—	+2.3	−1.6
Significance of Difference (P)	—	**<0.01**	**<0.01**	—	0.77	0.87	—	**<0.01**	**<0.01**

* These data represent the group means calculated from the average values of EHBF, arterial pressure and HPR for each patient, shown in detail in Table I. Non-equilibrium observations (*i. e.*, those prior to four minutes after a change in posture, as well as those associated with a faint) were deleted from the calculations.

† The significance of the differences was calculated by Fisher's method for the significance of unique samples (12). P represents the probability that a difference may be due to chance. Values of 0.05 or less (in bold type) denote a "significant" difference, and 0.01 or less a "highly significant" one.

‡ The differences refer to the change from the immediately preceding posture. Slight discrepancies between the mean changes from upright back to horizontal as compared to the mean values in these positions are due to the fact that observations were made on one hypertensive patient (Ric) during the control and upright periods, but not during recovery.

§ Average of the systolic and diastolic pressures.

hand in ice water for one minute caused slight or negligible effects upon EHBF, although it usually caused a temporary elevation of arterial pressure (Figure 3) and HPR. It was felt that this stimulus, despite its severity, was too brief in duration to produce regular effects upon EHBF; consequently, it was abandoned.

DISCUSSION

The results reported above confirm the impression gained from indirect evidence that active vasoconstriction occurs in splanchnic organs in addition to the kidneys when human subjects are tilted into the upright position. The decreases in EHBF found in the upright position can not be explained by changes in arteriovenous pressure gradient because the arterial pressure did not decrease proportionately and the atrial venous pressure is known not to increase in this position (7). Neither can the decreases in EHBF be attributed to other passive hydrostatic effects such as changes in blood volume (8) or intra-abdominal pressure (9). That the decreases in EHBF in the upright position are probably due to active vasoconstriction of sympathetic nervous origin has been indicated by repeating the studies of EHBF in some of the same subjects after splanchnicectomy (10).

No consistent differences in EHBF were found between the small group of hypertensive patients here reported and a larger group of normotensive subjects (11). The data indicate that the splanchnic circulation is not increased in hypertensive patients, but that calculated HPR is increased, reflecting the generalized vascular hypertonus usually regarded as characteristic of the disease. More evidence is being accumulated concerning these points by comparing the EHBF and HPR in the resting horizontal position in larger groups of hypertensive and normotensive subjects and will form the subject of a later report (11).

SUMMARY

Hepatic blood flow, as estimated by the bromsulfalein extraction method, decreased in eight normotensive and 12 hypertensive subjects after passive tilting into the upright (75°) position. It failed to change regularly in six subjects after immersion of a hand in ice water for one minute.

ACKNOWLEDGMENTS

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THE EFFECT OF SPLANCHNIC SYMPATHECTOMY IN HYPERTENSIVE PATIENTS UPON ESTIMATED HEPATIC BLOOD FLOW IN THE UPRIGHT AS CONTRASTED WITH THE HORIZONTAL POSITION [1]

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INTRODUCTION

It has been found that when normotensive or hypertensive subjects are tilted passively into an upright position estimated hepatic blood flow (EHBF) (2) decreases and calculated hepatic portal resistance (HPR) increases (3). These changes were assumed to be due to active splanchnic vasoconstriction, probably mediated over the splanchnic sympathetic nervous system. Direct evidence bearing on this assumption became available in some of the hypertensive patients undergoing splanchnic sympathectomy and subsequently studied by the same methods as before operation. Such studies are the basis of the present report.

METHOD

The method was identical with that used in the preoperative studies already reported (3). The first postoperative study was usually done within two weeks after the completion of the second stage of the splanchnicectomy, and, if possible, a second within 12 months. In addition, a few patients were studied one to nine years after (but not before) operation. The type of sympathectomy, unless otherwise noted in Table I, was the lumbodorsal (thoracolumbar) splanchnicectomy of Smithwick (4). After operation some patients [3] were unable to stand for any considerable length of time at a 75° tilt without marked postural hypotension and symptoms of faintness. Therefore, they were tilted back either to less marked angles (as noted in Table I) that they could tolerate long enough for reliable observations to be made, or to the horizontal position where paired samples of blood were drawn immediately for measurement of EHBF which, because of the time lag previously found to occur in this situation (3), was related to the upright period just before the tilt-back.

RESULTS

After lumbodorsal splanchnicectomy postural hypotension occurred to some extent in almost all the patients, particularly early after the operation. If it appeared suddenly it resulted not only in less accurate measurements of arterial pressure (which varied from moment to moment as the patient sighed or moved), but also in other technical difficulties, since it made it necessary hurriedly to terminate the period of upright posture, rather than leisurely to proceed until a more steady state of EHBF had been achieved. For these reasons the results in these instances were considered less reliable as equilibrium values than those observed when the patient was able to stand without great difficulty for a considerable period of time.[4]

In all cases after splanchnicectomy there were more nearly proportional decreases of EHBF and mean (one-half systolic plus diastolic) arterial pressure in the upright position (Table I) than before operation when relatively large reductions of hepatic blood flow and small changes of arterial pressure were usually found (Table II). Therefore, after operation calculated hepatic portal resistance (HPR) was often unchanged in the upright position whereas before operation it was usually increased. Thus, comparing average equilibrium figures, HPR in eight splanchnicectomized hypertensive patients increased on tilting from 6.8 to only 7.2, or 6% (which is not significant statistically), whereas in nine unoperated hy-

[1] Presented in part May 5, 1947 at the Thirty-Ninth Annual Meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey (1).

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[3] No patient was allowed to wear his abdominal pressure girdle or elastic stockings during the test.

[4] Only those data obtained after four minutes in a given position and without fainting are included in the statistical analysis in Table II.

TABLE I

Effects of the upright posture upon estimated hepatic blood flow (EHBF), mean (half systolic plus diastolic) arterial pressure, calculated hepatic portal resistance (HPR) and pulse rate in splanchnicectomized hypertensive patients, listed in order of the relative completeness of the sympathectomy, as judged by anatomic and physiologic criteria (4–6)

Patient Age Sex Surface area (*sq. m.*)	Time after operation	Horizontal position, Control period				Upright position						Horizontal position, Recovery period				
		EHBF (*cc./min./1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/cc./sec./1.73 sq. m.*)	Pulse rate (*Beats/min.*)	Minutes after tilt-up	Degree of tilt	EHBF (*cc./min./1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/cc./sec./1.73 sq. m.*)	Pulse rate (*Beats/min.*)	Minutes after tilt-back	EHBF (*cc./min./1.73 sq. m.*)	Mean arterial pressure (*mm. Hg*)	HPR (*mm. Hg/cc./sec./1.73 sq. m.*)	Pulse rate (*Beats/min.*)
Sta		1,336	126	5.7	69	3	75°	878	79	5.4	74	4	969	114	7.1	69
44	2 weeks*	970	125	7.7	69	6	75°	780	75	5.8	72	12	995	124	7.5	66
M		—	—	—	—	13	55°	490	66	8.1	72	21	1,135	122	6.4	66
1.91												30	1,174	123	6.3	69
Gol		1,005	177	11.0	84	5	60°	726	143	12.0	90	6	1,246	178	8.6	84
31	9½ weeks	1,120	184	9.9	84	9	60°	875	149	10.0	84	11½	1,228	177	8.6	88
M		1,381	181	7.9	92	15	60°	977	147	9.0	90	—	—	—	—	—
1.81																
		1,214	159	7.9	70	7	50°	1,267	150	7.1	94	5½	1,956	172	5.3	87
	7 months	1,366	169	7.4	76	15	40°	1,200	151	7.6	90	11	1,888	167	5.3	82
		1,374	171	7.5	74	20½	40°	1,276	160	7.5	91	—	—	—	—	—
Pau		2,330	121	3.1	72	4	75°	1,792†	71	2.4	?	5½	1,260	127	6.0	72
36	2 weeks	—	—	—	—	—	—	—	—	—	—	11½	1,678	124	4.4	78
M		—	—	—	—	—	—	—	—	—	—	22½	1,774	127	4.3	72
1.84																
Max		1,168	107	5.5	60	4	75°	899	77	5.1	120	5	1,496	105	4.2	58
43	2 weeks	1,435	112	4.7	58	7	60°	475‡‖	71	9.0	?	11	1,547	109	4.2	65
M		—	—	—	—	—	—	—	—	—	—	20	1,300	116	5.4	58
2.21		—	—	—	—	—	—	—	—	—	—	30½	1,255	115	5.5	66
McC		3,510	158	2.7	90	3½	75°	1,455	131	5.4	120	4	1,816	162	5.3	96
43	2 weeks	2,273	166	4.4	96	7½	75°	2,434	133	3.3	114	11½	2,938	159	3.3	90
M		—	—	—	—	—	—	—	—	—	—	24	1,790	159	5.3	96
1.75		—	—	—	—	—	—	—	—	—	—	32	2,727	167	3.7	96
		1,623	177	6.5	68	3½	75°	1,126	150	8.0	96	5	2,339	164	4.2	64
		1,908	176	5.5	68	9	60°	974	142	8.8	80	14½	2,363	154	3.9	72
	6 months	1,512	179	7.1	68	14	45°	1,069	144	8.1	80	23	1,619	158	5.9	72
		—	—	—	—	20	45°	1,352	151	6.7	88	32	2,029	166	4.9	72
She		1,813	163	5.4	84	3	75°	2,860†	104	2.2	108	6½	1,535	182	7.1	84
51	2 weeks	—	—	—	—	—	—	—	—	—	—	11½	1,525	160	6.3	88
M		—	—	—	—	—	—	—	—	—	—	18	1,757	162	5.5	90
2.02																
		1,568	132	5.1	68	9	45°	1,072	121	6.8	84	10	1,411	122	5.2	72
	4 months	1,384	134	5.8	68	13	45°	831	126	9.1	100	—	—	—	—	—
		1,234	134	6.5	68	19	45°	939	117	7.5	88	—	—	—	—	—
						25	45°	1,062	109	6.2	84	—	—	—	—	—

TABLE I—*Continued*

Patient Age Sex Surface area (sq. m.)	Time after operation	Horizontal position, Control period				Upright position						Horizontal position, Recovery period				
		EHBF (cc./min./1.73 sq. m.)	Mean arterial pressure (mm. Hg)	HPR (mm. Hg/cc./sec./1.73 sq. m.)	Pulse rate (Beats/min.)	Minutes after tilt-up	Degree of tilt	EHBF (cc./min./1.73 sq. m.)	Mean arterial pressure (mm. Hg)	HPR (mm. Hg/cc./sec./1.73 sq. m.)	Pulse rate (Beats/min.)	Minutes after tilt-back	EHBF (cc./min./1.73 sq. m.)	Mean arterial pressure (mm. Hg)	HPR (mm. Hg/cc./sec./1.73 sq. m.)	Pulse rate (Beats/min.)
Dea		1,867	159	5.1	81	4	75°	1,328	136	6.1	99	3	652‖	147	14.0	84
54	2 weeks	2,286	150	3.9	78	7	75°	2,418	138	3.4	102	9½	2,044	171	5.0	87
M		—	—	—	—	—	—	—	—	—	—	16½	2,615	156	3.6	84
1.82																
		1,477	176	7.1	66	7	75°	1,113	175	9.4	78	11	1,569	176	6.7	72
		1,545	175	6.8	69	11	75°	1,416	157	6.6	79	18	1,461	169	6.9	66
	7 months	1,399	185	7.9	72	16½	75°	1,270	164	7.7	83	23	1,275	182	8.6	72
		—	—	—	—	21½	75°	1,304	165	7.6	84	—	—	—	—	—
O'Le		1,289	126	5.9	81	3½	75°	745†	?	?	?	7	874	129	8.9	82
47	9 years	1,403	127	5.4	80	—	—	—	—	—	—	15½	1,004	120	7.2	75
F		—	—	—	—	—	—	—	—	—	—	21½	1,505	118	4.7	75
1.56																
Lee		779	106	8.2	60	20	75°	791	108	8.2	90	5½	839	116	8.3	60
35	4 years	891	105	7.1	52	25	75°	746	108	8.7	90	13	814	111	8.2	68
M		—	—	—	—	—	—	—	—	—	—	21	902	111	7.4	68
2.21																
Yof																
42	1 year	1,152	137	7.1	75	6	75°	1,184†	?	?	?	11	1,122	130	7.0	75
F		1,199	137	6.9	75	—	—	—	—	—	—	27	1,250	127	6.1	74
1.68																
Bar		943	143	9.1	60	4½	75°	780	124	9.5	104	5½	623	145	14.0	51
52	3 years	908	149	9.8	55	—	—	—	—	—	—	11½	736	141	12.0	69
F		834	154	11.0	71	—	—	—	—	—	—	20½	672	146	13.0	67
1.85																
Dun		2,378	108	2.7	84	3½	75°	690	88	7.7	155	4	884	130	8.8	86
30	2 weeks§	2,058	108	3.2	89	—	—	—	—	—	—	15½	1,218	98	4.8	92
M		1,687	111	4.0	93	—	—	—	—	—	—	—	—	—	—	—
1.82																

* Patient previously had had an incomplete splanchnicectomy elsewhere; present operation was a total thoracic extension (Rt = D1–D9; Lt = D1–D8) with bilateral excision of greater splanchnic nerves.

† Patient faint, tilted back, sample for EHBF taken immediately.

‡ Patient faint, sample for EHBF taken, tilted back immediately.

‖ This value was obtained during a rapid rise (greater than 0.0006 mg./cc./min.) in the peripheral venous serum concentration of BSP. It is questionable and is deleted from the statistical analysis (Table II).

§ Patient had a supradiaphragmatic splanchnicectomy (Rt = D7–D12; Lt = D8–D12, inclusive).

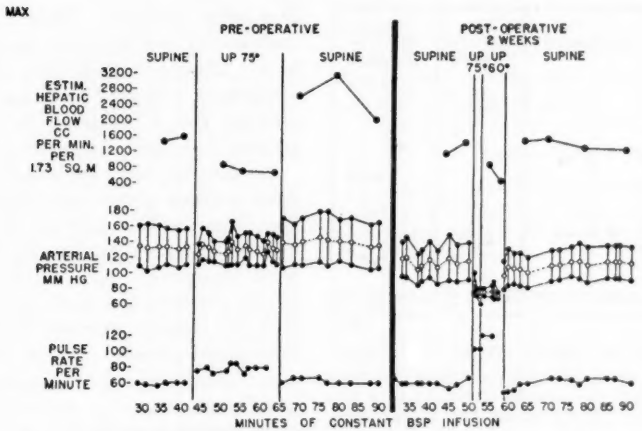

FIG. 1. CHART OF EHBF, ARTERIAL PRESSURE AND PULSE RATE OF A HYPERTENSIVE PATIENT (MAX), STUDIED IN THE HORIZONTAL AND UPRIGHT POSITIONS BEFORE AND AFTER A LUMBODORSAL SPLANCHNICECTOMY

"Mean" (half systolic plus diastolic) arterial pressure is indicated by the open circles and interrupted lines. During and after the upright position in the preoperative test, this patient had a marked anxiety reaction that began with a severe headache.

pertensive patients it increased from 7.3 to 10.1, or 38% (which is highly significant statistically).

The findings in the splanchnicectomized group may also be demonstrated by charts of experiments done on individual cases before and after operation (Figures 1, 2). Here the reductions of EHBF

TABLE II

The effect of splanchnicectomy on the mean responses of the estimated hepatic blood flow (EHBF), arterial pressure and hepatic portal resistance (HPR) to upright tilting of the body*

	EHBF (*cc./min./1.73 sq. m.*)			Mean§ arterial pressure (*mm. Hg*)			HPR (*mm. Hg/cc./sec./1.73 sq. m.*)		
	Horizontal (Control)	Upright	Horizontal (Recovery)	Horizontal (Control)	Upright	Horizontal (Recovery)	Horizontal (Control)	Upright	Horizontal (Recovery)
Splanchnicectomized patients (11 experiments on 8 patients)									
Mean	1,472	1,171	1,521	149	125	148	6.8	7.2	6.6
Standard Error of Mean	176	163	172	8	9	8	0.6	0.6	0.8
Mean Difference†	—	−301	+360	—	−24	+23	—	+0.4	−0.5
Significance of Difference (P)‡	—	**<0.01**	**<0.01**	—	**<0.01**	**<0.01**	—	0.09	0.44
Unoperated hypertensive patients (n = 9)									
Mean	1,357	960	1,417	158	157	155	7.3	10.1	7.2
Standard Error of Mean	97	68	172	7	7	8	0.6	0.7	0.7
Mean Difference†	—	−397	+428	—	−1	0	—	+2.8	−2.4
Significance of Difference‡	—	**<0.01**	**<0.05**	—	0.78	0.97	—	**<0.01**	**<0.01**

* The mean values of EHBF, arterial pressure and HPR for the group were calculated from the averages of the observations on each individual. See Table I for details of splanchnicectomized patients, and the preceding paper (3) for unoperated hypertensive patients.

† Differences refer to the changes from the immediately preceding position.

‡ The significance of the difference was calculated by the method of Fisher for unique samples (8). P values of 0.05 or less (bold type) denote "significant" differences, and values of 0.01 or less "highly significant" differences.

§ Average of systolic and diastolic pressures.

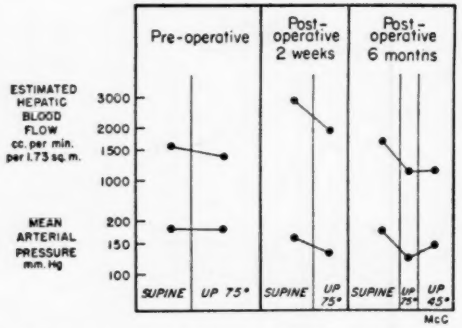

FIG. 2. CHART OF AVERAGE EHBF AND "MEAN" (HALF SYSTOLIC PLUS DIASTOLIC) ARTERIAL PRESSURE IN THE HORIZONTAL AND UPRIGHT POSITIONS IN A HYPERTENSIVE PATIENT (McC) BEFORE, TWO WEEKS AFTER, AND SIX MONTHS AFTER LUMBODORSAL SYMPATHECTOMY

in the upright position before operation are seen to be essentially unrelated to changes in arterial pressure, whereas after operation they were usually associated with sizeable decreases in arterial pressure.

After return to the horizontal position the EHBF in post-operative, as in preoperative, patients usually returned toward, to, or above the previous horizontal control values. Arterial pressure recovered from postural hypotension and calculated HPR did not change significantly (Table II).

DISCUSSION

The results reported here indicate that the splanchnic sympathetic nervous system of hypertensive patients probably mediates, at least in part, the vasoconstrictor response in the hepatic portal circulation to the upright posture. Thus, sizeable increases in HPR usually did not occur in the upright position after splanchnicectomy, whereas in the same subjects before operation and in normotensive control subjects (3) they did occur. Obviously this does not prove that the changes in EHBF found after splanchnicectomy were only passive and due solely to associated changes in arterial pressure. However, since the decreases in EHBF in the upright position were no greater after than before operation, whereas the reductions in arterial pressure were much greater, one may assume that there was relatively much less active vasoconstriction in the hepatic-portal region after than before operation. These results give direct confirmation to independent hemodynamic studies in this laboratory which have shown that reflex vasopressor responses to certain blood-pressure-lowering stimuli, including the upright posture, are greatly reduced after splanchnicectomy (5, 6).

Besides elucidating the influence of the sympathetic nervous system upon hepatic-portal blood flow, these studies indicate the importance of hydrostatic factors, particularly of orthostatic arterial hypotension, upon EHBF. Decreases in arterial pressure, if sizeable, were usually associated with sizeable reductions of EHBF in sympathectomized patients. On the other hand, when minor changes of arterial pressure such as those usually encountered in normotensive or unsympathectomized hypertensive patients in the upright position occurred in splanchnicectomized patients they were associated with little change in EHBF.

Although for the reasons just given, both the activity of splanchnic sympathetic nervous system and the level of the arterial pressure appear to be important factors in the normal regulation of EHBF, other influences also must be assumed to play a role. Thus, even in well-sympathectomized patients, major "spontaneous" changes in EHBF occurred, particularly during and after orthostasis, that could not be accounted for on the basis of the first two factors alone. For example, after standing upright and being returned to the horizontal position some postoperative patients (Gol, McC, and Dea) had large increases in horizontal EHBF which could not be explained by rises in arterial pressure.

The observations made in patients during orthostatic syncope are also of interest in this connection. As mentioned in a previous paper (3), the tendency to collapse in the upright position before operation seemed usually related directly to the absolute level to which hepatic-portal blood flow fell. Thus, when EHBF fell to 750 cc./min. and failed to recover, collapse was usually imminent even though the arterial pressure up to that point had been well-sustained. After splanchnicectomy, however, while patients in the upright position usually had a lower arterial pressure and a proportionate decrease in EHBF (at times below 750 cc./min.), the expected circulatory "decompensation" characteristic of vaso-vagal syncope did not always appear (notably in Sta). On the other

hand, three splanchnicectomized patients (Pau, She and Yof) fainted while EHBF remained good and calculated HPR actually decreased.

The results of these studies provide probably valid indications of the physiologic role of the intact splanchnic sympathetic nervous system, not only in hypertensive but perhaps also in normotensive individuals with respect to the regulation of hepatic-portal blood flow. Along with other observations in this laboratory, they suggest that the sympathetic nervous system normally plays a vasoconstrictor role in the hepatic-portal circulation. The vasoconstrictor and vasodilator influences in this circuit may be presumed usually to be balanced. Conceivably, if they became unbalanced through either overactivity or inhibition of one as compared with the other, blood flow and calculated hepatic-portal resistance would change. Thus, it is possible that the early decrease and late return of increased HPR in resting horizontal hypertensive patients after splanchnicectomy (7) is due to an early unopposed, though normal, vasodilator activity followed by a moderation of that activity or by a return of "intrinsic vasoconstrictor tone," or both. Furthermore, it is possible that the sizeable "spontaneous" variations in EHBF observed after splanchnicectomy may be due to greater or lesser amounts of the remaining "vasodilator activity."

SUMMARY AND CONCLUSIONS

After splanchnic sympathectomy, reductions of hepatic blood flow in hypertensive patients tilted into the upright position are associated with sizeable decreases in arterial pressure and little change in average hepatic-portal resistance, whereas before operation reductions in EHBF in the upright position occur with little change in pressure and increases in HPR. It is concluded that the splanchnic sympathetic nervous system mediates the hepatic-portal vasoconstrictor response to the upright posture in hypertensive patients and probably also in normal subjects.

ACKNOWLEDGMENTS

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ADRENOCORTICAL CONTROL OF SODIUM AND POTASSIUM EXCRETION IN THE NEWBORN PERIOD[1]

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The role of the adrenals in response to disease during the neonatal period has long been a subject of speculation. The anatomical peculiarities of the adrenal cortex in this period are well known. Jaudon (1) has suggested that transient deficiencies in adrenocortical function occur in the newborn period. However, few experimental studies have been reported. Venning (2) has reported that the newborn under one week of age excretes less neutral reducing lipids in response to the administration of adrenocorticotropic hormone (hereinafter called ACTH) than does the older infant. We have found that during the first week of life the decrease in circulating eosinophils after the administration of ACTH is less than in older infants (3).

The author has suggested that stimulation of the newborn adrenal does not cause sodium retention (4). In the present study ACTH was given to newborn infants to study the effect of the adrenal cortex on electrolyte control.

METHODS

Eleven infants in the premature nursery of the Harriet Lane Home and three full term infants three weeks of age or under and one six months old full term infant on the general ward of the Harriet Lane Home were studied during the winter of 1949–50. The premature infants were all healthy with the exception of two who were being treated for retrolental fibroplasia.[2] The six months old infant was being observed in the hospital for congenital nonhemolytic jaundice. Two of the other three patients on the infants ward had recovered from the acute pneumonitis for which they were admitted. The third patient had congenital glaucoma.

Complete urine collections were made with the few exceptions to be noted in the tables. Soft rubber tubing fitting snugly over the penis was used for collecting urine from the males. Urine was collected from female patients by placing the infant in an ordinary bassinet with the head of the bassinet slightly elevated, and with the patient's buttocks over a Buchner funnel. The patients were supported in this position by slings supporting their lower thighs. Five cc. of 25% acetic acid were added to each day's urine collection.

Patients were weighed routinely three times a week on the regular balances of the wards and nursery. Weights are recorded for the beginning of the daily periods.

Eosinophil counts were made by the method of Randolph (5). Sodium and potassium in the urine were measured by an internally compensated Perkin-Elmer flame photometer. 17-Ketosteroids and neutral reducing lipids were measured by methods previously described (6). Nitrogen in the urine was determined by nesslerization. Serum and stool electrolytes were not determined. Dietary intake was calculated from conventional tables and random samples were analyzed for sodium and potassium content using the wet ashing method of Wallace (7) and measuring with a flame photometer. The volume of feeding mixture in each bottle was accurately measured, but when any of the bottle was refused, the amount was estimated by the nurse using the calibrations on the bottle. Fortunately, this occurred rarely.

ACTH[3] was given to 11 patients for one day only, one receiving 10 mg. intramuscularly every six hours, one 10 mg. every eight hours, six 5 mg. every six hours, two 2 mg. every six hours, and one 1 mg. every six hours. Two other patients received ACTH four times daily for periods of seven and 10 days respectively.

When desoxycorticosterone acetate (hereinafter called DCA) or cortisone were used they were given as a single intramuscular injection at the beginning of the daily period.

RESULTS

Seven of the newborn infants tested with ACTH for one day were prematures who had weighed between 1,210 and 1,800 grams at birth (Cases 1–4, 10, 11, 14). At the time of the study their weights were between 1,330 and 3,125 grams.

[1] This work was made possible by a grant from the American Cancer Society for studies on the relationship of the pituitary hormones, thyroid hormone, and the steroid hormones of the adrenal glands and gonads to normal and abnormal growth, and by a grant from the Commonwealth Fund for the study of endocrine problems in childhood.

[2] These were treated under the supervision of Drs. W. and E. Owens.

[3] Kindly supplied by Dr. J. R. Mote of the Armour Laboratories, Chicago, Illinois.

The oldest was 41 days old. In addition three full term infants three weeks of age or less and weighing between 3,150 and 4,150 grams were studied. Sodium retention on the day of ACTH administration in these cases was minimal or non-existent. However, in all but one of the patients there was a significant increase in excretion of sodium in the urine on the day following administration of ACTH. This loss in the urine was often greater than the calculated intake for the day. It was accompanied by an increase in urinary volume. There was usually a decreased rate of the weight gain in this period. The one infant who did not show this sodium loss was one of those receiving the smallest doses of ACTH.

The effect of ACTH on potassium excretion was more variable. In some infants (and these usually were among the larger who received the larger doses) there was an increased urinary excretion of potassium on the same day that ACTH was given whereas there was no change in urinary sodium until the following day. In other infants, potassium excretion did not change or else varied directly with the volume of urine. The initial rise in potassium excretion was invariably associated with increased urinary nitrogen excretion. Usually the amount of nitrogen excretion did not indicate that there was sufficient protein catabolism to account for the potassium lost. In several cases there was a secondary rise in potassium excretion two or three days after ACTH was given. This, too, was always associated with a secondary rise in nitrogen excretion.

The nitrogen excretion in the urine showed a uniform rise when ACTH was administered. This occurred either on the day ACTH was given or on the following day.

In all those infants over one week of age on whom counts were made, there was a fall in circulating eosinophils irrespective of the dose of ACTH. Since these results were in accord with previous work (3), such counts were made only in the first few studies. It is of importance to note that there was the expected rise in excretion of 17-ketosteroids and neutral reducing lipids after administration of ACTH. On continued stimulation, the 17-ketosteroid levels rose at times to normal adult female levels and the neutral reducing lipids far exceeded normal adult levels.

Since in these newborns ACTH failed to cause sodium retention which usually occurs in older children and adults, it is important to note the effect of DCA in this group of patients. The two newborns who were given 5 mg. of DCA in a single intramuscular injection and the one who was given 2 mg. showed a definite decrease in urine volume and sodium excretion (Cases 9–11). The patient who received only 1 mg. of DCA showed no definite sodium retention although his urine volume did decrease. DCA caused no increase in potassium excretion, but instead, in some cases, there was a decrease parallel to the reduction in urine volume.

Two premature infants were given cortisone (Cases 13, 14). When 50 mg. were given to the first infant there was no significant change in electrolyte excretion. The second infant who received 25 mg. of cortisone showed marked urinary retentions of water, sodium and potassium. This

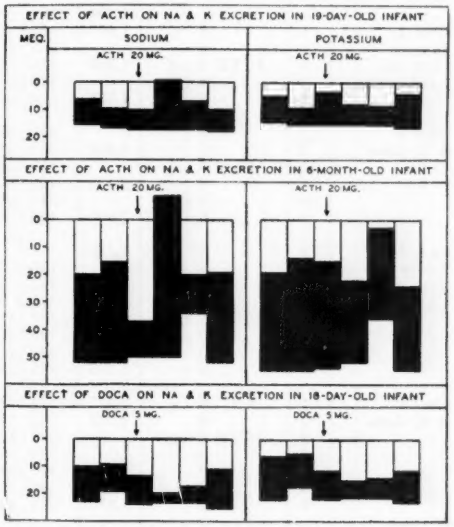

FIG. 1. ELECTROLYTE BALANCES OF A 19 DAY OLD INFANT GIVEN ACTH, AND OF A SIX MONTH OLD INFANT GIVEN ACTH AND AN 18 DAY OLD INFANT GIVEN DCA

Each column represents one day. Intakes are plotted down from the zero line, and urinary excretion (blacked in) is plotted up from the intake. The resultant white spaces below the zero line represent positive balances and the blacked in areas above the zero line represent negative balances. No allowance is made for stool or sweat electrolytes.

infant then was given ACTH and responded to it in the same manner as the other newborns.

In addition to the ten infants who were treated with ACTH for only 24 hours, two premature infants were treated with ACTH for seven and 10 days respectively in an experimental attempt to influence retrolental fibroplasia (Cases 7, 8). Neither of these patients showed any signs of escape from the effects of ACTH on electrolyte balance. The increased sodium excretion continued for two or three days after discontinuing ACTH. One of these prematures developed diarrhea while being treated with ACTH (Case 8). He continued to pour out more sodium in spite of the fact that there was probably an increased loss of sodium in the stools. His weight fell sharply and it was felt that the magnitude and rapidity of the loss were greater than would be expected from the relative mildness of his diarrhea. Three days after ACTH was discontinued conservation of sodium occurred due to a decrease in the urinary excretion. Until this time the additional amounts of parenteral saline received were excreted within 24 hours in the urine. He was able to retain potassium better even while getting ACTH, although one day while he was having diarrhea the urinary excretion exceeded the intake. The urinary volume remained high until one week after

TABLE I

Electrolyte and steroid data for 15 infants studied

Case	Day	Rx	Wt.	Intake			Urinary output				Urinary steroids	
				Vol.	Na	K	Vol.	Na	K	N	17KS	Neutral red. lipids
			grams	*cc.*	*meq*	*meq*	*cc.*	*meq*	*meq*	*grams*	*mg.*	*mg.*
Case 1	1	0	1,600	82	5.5	4.6	84	4.5	3.8	0.29	0.54	0.34
18 days	2	0		205	13.9	11.5	72	4.8	4.2	0.34	0.44	0.31
birth wt.	3	ACTH 4 mg.	1,660	210	14.3	11.7	70	4.8	5.5	0.38	0.94	0.57
1,765 grams	4	0		210	14.3	11.7	65	5.2	3.5	0.32	0.54	0.56
	5	0	1,775	210	14.3	11.7	54	3.1	3.1	0.17	0.32	—
Case 1	1	0		430	18.0	16.9	incomplete collection					
at 33 days	2	0	2,400	510	18.0	16.9	240	6.7	6.5	0.87	0.85	0.29
	3	ACTH 4 mg.		510	18.0	16.9	240	8.6	8.9	1.45	1.43	0.28
	4	0	2,465	450	18.0	16.9	203	11.8	9.1	0.13	1.22	0.28
	5	0		510	18.0	16.9	194	7.4	7.4	0.08	0.86	0.26
	6	0	2,600	485	18.0	16.9	238	8.1	8.8	0.67	0.97	0.29
Case 2	1	0	1,330									
9 days	2	0		126	8.3	7.0	60	3.1	3.0	0.27	0.01	0.06
birth wt.	3	0	1,345	146	9.6	8.0	46	3.2	4.3	0.31	0.00	0.06
1,430 grams	4	0		150	9.9	8.3	50	2.5	4.3	0.26	0.04	0.05
	5	ACTH 8 mg.	1,430	175	11.5	9.6	60	3.3	5.4	0.30	0.50	0.12
	6	0		180	11.9	9.9	110	11.2	4.1	0.41	0.39	0.14
	7	0		180	11.9	9.9	84	7.2	5.6	0.31	0.00	0.11
	8	0	1,495	205	13.5	11.5	33	2.8	6.3	0.38	0.37	0.08
Case 2	1	0	2,125	375	15.0	13.9	188	7.1	11.1	0.99	0.56	0.10
at 41 days	2	0	2,235	345	13.8	12.8	152	6.4	9.9	0.97	0.58	0.12
	3	ACTH 8 mg.		370	14.8	13.7	218	11.1	12.6	1.22	0.87	0.22
	4	0		390	15.6	14.4	200	13.6	9.8	1.64	0.80	0.14
	5	0	2,340	360	14.4	13.4	160	7.0	13.0	0.94	0.90	0.13
	6	0		390	15.6	14.4	150	6.6	9.0	0.88	0.90	0.12
	7	0	2,450	390	15.6	14.4	170	8.3	13.1	0.71	0.62	—
Case 3	1	0	2,200	350	13.5	13.0	185	6.1	4.1		0.76	0.21
11 days	2	0		345	13.8	12.8	170	5.4	3.9		0.60	0.18
birth wt.	3	ACTH 8 mg.	2,500?	350	14.0	13.0	142+	5.7	4.8		1.00	0.25
2,100 grams	4	0		355	14.2	13.2	155	5.4	3.4		0.88	0.17
	5	0	2,450	375	15.0	13.9	165	6.9	5.1		0.92	0.19
Case 4	1	0	2,320	390	15.6	14.4	150	9.5	9.7	0.55	0000	—
19 days	2	0		415	16.6	15.4	115	6.9	6.7	0.49	0000	0.18
birth wt.	3	ACTH 20 mg.	2,400	420	16.8	15.6	150	6.4	12.3	1.03	1.20	0.23
1,800 grams	4	0		420	16.8	15.6	195	17.0	7.8	0.97	0.72	—
	5	0		420	16.8	15.6	130	10.0	7.5	0.72	0000	—
	6	0	2,455	445	17.8	16.5	175	7.7	12.1	1.02	0000	0.14

TABLE I—*Continued*

Case	Day	Rx	Wt.	Intake			Urinary output				Urinary steroids	
				Vol.	Na	K	Vol.	Na	K	N	17KS	Neutral red. lipids
			grams	*cc.*	*meq*	*meq*	*cc.*	*meq*	*meq*	*grams*	*mg.*	*mg.*
Case 5	1	0	3,150	550	22.0	20.6	170	11.8	14.6	0.83	0.80	
21 days	2	0	3,140	580	23.3	21.8	195	13.2	15.4	0.87	1.10	
birth wt.	3	ACTH 20 mg.		555	22.3	20.8	205	8.8	18.0	1.05	2.40	
2,800 grams	4	0	3,220	600	24.1	22.5	295	30.0	13.8	1.67	1.80	
	5	0		590	23.7	22.1	265	20.6	18.3	1.14	0.74	
	6	0	3,460	530	21.3	19.4	150	14.1	25.8	1.78	0.66	
Case 6	1	0	3,500	360	14.4	13.3	45	3.6	3.6			
17 days	2	0		585	20.4	21.5	107	6.6	7.7			
birth wt.	3	ACTH 20 mg.	3,500	580	22.6	24.8	160	6.9	18.8			
3,209 grams	4	0	3,500	605	22.6	22.5	185	13.9	9.6			
	5	0		600	17.6	18.6	270	11.6	16.2			
Case 7	1	0	3,125	600	24.0	22.4	110	5.4	6.4	0.39	0.90	0.30
69 days	2	ACTH 20 mg.		600	24.0	22.4	200	6.2	15.6	0.97	0.90	0.35
birth wt.	3	20		600	24.0	22.4	320	14.7	19.6	1.73	—	—
1,600 grams	4	20	3,225	600	24.0	22.4	340	14.6	16.0	2.74	5.50	—
	5	20		600	24.0	22.4	incomplete collection, concentrations of same order					
	6	20	3,385	600	24.0	22.4	of magnitude as the previous days for both days					
	7	ACTH 40 mg.		600	24.0	22.4	240	22.6	15.1	1.82	—	—
	8	40	3,480	600	26.9	23.4	325	32.5	12.7	—	7.20	2.16
	9	0		585	26.9	22.4	240	33.0	7.2	1.56	—	—
	10	0		600	28.0	25.6	350	37.8	16.0	1.04	2.00	—
	11	0	3,270	600	28.0	25.6	150	5.0	15.0	—	—	—
	12	0		600	28.0	25.6	140	11.2	9.5	1.14	0.90	—
	13	0	3,500									
Case 8	1	0	2,155	360	14.4	13.3	105	6.5	6.3	0.68	—	—
52 days	2	0	2,205	360	14.4	13.3	155	7.8	6.3	0.80	1.60	—
birth wt.	3	0		360	14.4	13.3	115	5.2	5.4	0.59	1.64	0.11
1,285 grams	4	ACTH 8 mg.	2,295	355	14.2	13.2	110	5.8	5.8	0.71	1.66	—
	5	ACTH 10 mg.		360	14.4	13.3	125	7.5	8.6	0.51	—	—
	6	10		380	15.2	14.1	158	9.5	5.9	0.95	2.40	—
	7	ACTH 20 mg.	2,220	440	17.6	16.3	no collection					
	8	20		450	18.0	16.7	210	17.4	6.9	2.10	5.80	0.50
	9	20	2,260	450	18.0	16.7	no collection					
	10	20		400	16.0	14.8	195	17.0	7.6	2.10	3.20	
	11	20	2,180	325	13.0	12.0	no collection					
	12	20	2,125	300	00	00	205	9.0	5.1	1.80	—	—
	13	20	2,045	400	3.0	00	no collection					
	14	0	1,945	425	21.8	9.1	185	7.9	1.0	1.00	—	—
	15	0	2,040	390	8.1	11.7	290	24.5	2.7	1.10	—	—
	16	0	2,090	440	10.9	13.8	188	0.6	6.0	0.71	0.50	—
	17	0	2,060	440	11.6	13.6	no collection					
	18	0	2,160	390	8.9	14.0	165	0.3	7.4	1.20	—	—
	19	0	2,280	390	9.2	14.6	no collection					
	20	0	2,360	390	11.9	17.8	88	2.2	6.5	0.29	—	—
	21	0	2,470									
Case 9	1	0	4,150	575	23.0	21.6	177	12.9	15.8	—		
18 days	2	0	4,170	465	18.8	17.5	118	9.8	12.3			
birth wt.	3	DCA 5 mg.		595	23.8	22.4	128	10.4	10.6			
3,800 grams	4	0	4,200	590	23.6	22.2	90	3.4	7.2			
	5	0		590	23.6	22.2	137	6.6	7.8	0.82	0.40	—
	6	0		625	25.0	23.5	215	13.5	12.4	1.36	0.50	0.18
	7	ACTH 40 mg.		685	27.3	25.6	240	10.8	18.0	1.75	0.60	0.48
	8	0	4,520	680	27.1	25.3	292	28.8	12.0	1.39	0.67	0.15
	9	0	4,510	670	26.8	25.1	290	13.9	21.2	2.21	0.60	—
	10	0	4,610	710	28.4	26.6	270	17.0	23.2	1.79	0.73	—

TABLE I—*Continued*

Case	Day	Rx	Wt.	Intake			Urinary output				Urinary steroids	
				Vol.	Na	K	Vol.	Na	K	N	17KS	Neutral red. lipids
			grams	*cc.*	*meq*	*meq*	*cc.*	*meq*	*meq*	*grams*	*mg.*	*mg.*
Case 10	1	0	1,560	210	10.9	8.2	60+	5.4	3.3			
21 days	2	0		210	10.9	8.2	96	7.2	4.3			
birth wt.	3	DCA 5 mg.	1,645	220	11.4	8.6	53	3.4	2.9			
1,210 grams	4	0		226	11.8	8.8	50	2.8	3.2			
	5	0		238	12.4	9.3	50	3.3	2.9			
	6	0	1,760	240	12.5	9.4	65	4.8	2.6		0.33	
	7	0		240	12.5	9.4	90	9.0	4.4		0.20	
	8	ACTH 20 mg.	1,850	260	13.5	10.1	60	4.4	6.1		0.73	
	9	0		270	14.1	10.8	150+	18.3	2.7		0.60	
	10	0	1,860	295	15.4	11.8	100	4.7	7.0		—	
	11	0	2,050									
Case 11	1	0	1,460	210	13.8	11.5	100	7.0	6.0	0.62	0.70	—
20 days	2	0		218	14.2	12.0	110	8.2	8.5	0.66	0.60	0.15
birth wt.	3	ACTH 20 mg.	1,560	230	15.2	12.6	115	7.4	8.0	0.73	0.60	0.26
1,250 grams	4	0		240	15.8	13.2	160	17.6	7.0	1.12	1.20	0.41
	5	0		250	16.4	13.7	98	8.1	5.7	0.62	0.40	—
	6	0	1,565	267	17.6	14.7	160	4.7	8.4	0.50	0.40	0.09
	7	DCA 2 mg.		270	17.8	14.8	125	8.0	9.0	0.75		
	8	0	1,655	270	17.8	14.8	73	5.9	9.0			
	9	0		270	17.8	14.8	68	7.3	5.2			
	10	0	1,715	270	17.8	14.8	78	8.4	5.8			
Case 12	1	0	2,130									
18 days	2	0		270	10.8	10.0	138	8.0	6.3			
birth wt.	3	0	2,195	320	12.8	11.9	145	8.2	9.4			
1,630 grams	4	0		330	13.2	12.2	165	9.0	9.4			
	5	DCA 1 mg.	2,325	320	12.8	11.9	100	7.2	6.8			
	6	0		325	13.0	12.0	164	11.0	9.0			
	7	0		330	13.2	12.2	150	8.4	8.7			
Case 13	1	0	-	265	13.9	10.5	84	6.0	5.6	0.27		
22 days	2	0	1,860	280	14.7	11.0	85	5.5	5.3	0.35	0.85	—
birth wt.	3	0		295	15.5	11.6	106	7.6	6.3	0.34	1.06	0.14
1,540 grams	4	Cortisone 50 mg.	1,940	290	15.3	11.4	93	7.0	6.3	0.36	1.14	0.13
	5	0		300	15.8	11.9	130	7.8	7.3	0.50	1.22	0.18
	6	0		300	15.8	11.9	155	9.9	7.4	0.49	0.93	—
	7	0	2,005	300	15.8	11.9	84	5.7	5.1	0.38	0.16	0.09
	8	0		295	15.5	11.6	118	7.3	6.7	0.46	0.06	—
	9	0	2,075									
Case 14	1	0	1,650	240	16.3	13.4	116	8.9	9.6	0.54	0.77	
25 days	2	0		240	16.3	13.4	110	7.5	8.3	0.46	0.70	0.08
birth wt.	3	Cortisone 25 mg.	1,735	240	16.3	13.4	40	2.9	3.0	0.22	{3 day aver.	{2 day aver.
1,455 grams	4	0		240	16.3	13.4	70	2.9	2.7	0.25	0.71	0.05
	5	0		265	18.0	14.8	73	3.5	4.5	0.26		
	6	0	1,825	270	18.3	15.1	111	7.5	7.2	0.44	0.84	{2 day aver.
	7	0		270	18.3	15.1	85	6.0	6.1	0.38	0.58	0.13
	8	ACTH 30 mg.	1,950	280	19.0	15.7	117	6.1	9.7	0.57	0.73	0.15
	9	0		300	20.4	16.8	120+	12.5	6.6	0.75	0.74	0.19
	10	0	1,965	325	22.0	18.2	120	12.4	7.0	0.53	0.64	—
	11	0		330	22.5	18.5	95	4.3	10.5	0.89	0.51	0.11
	12	0		330	22.5	18.5	117	9.1	10.3	0.78	0.63	—
	13	0	2,025	330	22.5	18.5	133	11.0	10.9	0.80	0.77	—
	14	0	2,105									
Case 15	1	0	7,020	1,025	52.0	55.0	370	32.0	36.0		0.70	
6 months	2	0		1,025	52.0	55.0	415	37.0	41.0		0.50	
birth wt.	3	ACTH 20 mg.		1,025	50.0	54.0	260	13.0	39.0		0.70	
3,500 grams	4	0	7,100	960	50.0	52.0	535	59.0	30.0		0.90	
	5	0		730	34.0	36.0	235	14.0	33.0		0.50	
	6	0		1,000	52.0	55.0	225	33.0	31.0		0.50	

ACTH was discontinued. Sodium concentration in the urine never exceeded 87 meq/l.

In comparison with the infants of the younger age group a study was made on one six month old full term infant (Case 15). He showed sodium retention when given ACTH for one day in a manner entirely similar to that shown by the usual adult receiving ACTH for one day.

Figures for the intake and excretion of ions and for the excretion of steroids and nitrogen in these cases are given in Table I. Figure 1 compares the electrolyte balance of a characteristic newborn given ACTH and also DCA with that of a six month old infant given ACTH.

DISCUSSION

The functional immaturity of the kidney of the infant is well known having been elucidated by McCance and Young (8), Rubin, Bruck and Rapoport (9), and others. However, it is difficult to account for this peculiar electrolyte response to ACTH on this basis since the administration of DCA to the newborn caused decreased excretion of sodium as it does in the older child and adult. This suggests that the kidney of the newborn is capable of responding normally to adrenocortical hormone. It seems more probable that the peculiarity of electrolyte excretion is due to a difference in the hormones secreted by the immature adrenal. This might be due either to the secretion of a hormone not present in later life or to an alteration in the balance between hormones having varying quantitative or qualitative effects on sodium excretion. The latter alternative might be considered on the basis of the theory advanced by Thorn and associates (10) suggesting that weak sodium retainers such as Compound E and F compete with potent sodium retainers such as DCA and thereby induce salt loss. Thus it is possible that in the case of the newborn the adrenal when stimulated by ACTH may secrete relatively more Compound F-like hormone than the adult adrenal under similar circumstances. In patients with congenital hyperplasia the ACTH-induced secretion of androgenic steroids might also compete with DCA-like steroids, and thereby induce the observed sodium loss (6, 11).

In our small series there was no demonstrable difference in response between premature and full term infants of the same post-natal age, but it is possible that the abnormal effects of the adrenal on electrolyte excretion persist longer in premature than in full term infants.

SUMMARY AND CONCLUSIONS

Twelve newborn infants were given ACTH for periods of one day to ten days. This was regularly accompanied by the expected increase in the excretion of neutral reducing lipid, 17-ketosteroids, and urinary nitrogen. The urinary excretion of potassium showed only moderate changes, usually increasing on the day of ACTH injection as expected from adult experience. Sodium excretion was always increased one day after an adequate dose of ACTH was given. This frequently was of sufficient magnitude to exceed the dietary intake of this ion. One six month old infant retained sodium after the administration of ACTH in the same manner as adults. When DCA was administered to newborns they reacted like adults, showing a decreased excretion of sodium. In one newborn, 25 mg. of cortisone produced sodium retention; 50 mg. of cortisone given to another newborn produced no effect on the sodium balance.

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STUDIES ON THE COMBINED USE OF MEASUREMENTS OF SWEAT ELECTROLYTE COMPOSITION AND RATE OF SWEATING AS AN INDEX OF ADRENAL CORTICAL ACTIVITY [1]

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INTRODUCTION

Since Conn and his associates (1, 2) proposed that the concentration of chloride or sodium in thermal sweat can be used as an index of that function of the adrenal cortex concerned primarily with electrolyte metabolism, studies concerning the factors which are related to or influence the electrolytic composition of sweat have gained greatly in significance. In the past, several investigators have obtained data which suggested that the concentration of chloride in sweat is positively correlated with the rate of sweating (3, 4) and skin temperature (5). However, the relations of these variables to sweat electrolyte composition have never been defined with precision nor have they been studied in connection with the effect of adrenal cortical activity on the composition of sweat.

The present paper is concerned initially with studies which show that certain non-endocrine factors play a major role in the determination of sweat electrolyte composition. A method for taking these factors into consideration in the utilization of sweat electrolyte measurements as an index of hormonal activity is described. Finally investigations of adrenal cortical function based on this newly developed index are presented.

METHODS

Unless otherwise noted subjects were maintained on a standard diet for three to five days prior to the first collection of sweat and throughout the interval during which the collections were made. The *standard* daily diet consisted of a low-salt diet, estimated to contain 1.5 gms. of sodium chloride, supplemented with weighed sodium chloride in such amounts as to bring the total sodium chloride intake up to 5 gms. per square meter of body surface (m^2) per day. Drinking water was allowed *ad lib.*

Sweating was induced by placing the subject in a room the temperature of which could be maintained at the desired level by automatic temperature regulators. Usually additional stimulation was provided by having the subject immerse one arm in a bath of warm water, the temperature of which was kept nearly constant at the value desired throughout the collection period.

Sweat was collected from the non-immersed arm by enclosing it in a loosely-fitting watertight envelope, made of rubber dental dam and fitted with a glass delivery tube at its lower end. Immediately before the envelope was placed in position, the skin of the arm was thoroughly cleaned with tap water, 70 per cent aqueous solution of ethanol and finally, distilled water.

Rates of sweating were expressed as ml. per hour per 0.1 m^2 of the skin surface enclosed in the envelope, the surface area of the skin surface being calculated according to the formula of DuBois (6). The volume of sweat was taken as the sum of the free sweat delivered from the envelope *plus* the amount adhering to the inner surface of the envelope. The latter was determined by weighing the envelope before and after it had been wetted with sweat.

Temperatures of the room, water bath and skin were recorded by a Micromax apparatus. Separate electrodes were used in the determination of room and bath temperatures and of the skin enclosed in the rubber envelope. For the latter determination two electrodes were employed, one being fixed on the lateral surface of the arm just above the elbow and one on the dorsal surface of the forearm just above the wrist. Readings from each electrode were recorded every eight minutes during the period of collection. The reported temperature in each instance is the average of the individual readings. In these experiments, the intensity of thermal stimulation (see below) was arbitrarily taken to be the room temperature when the bath temperature was kept the same

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(44.5 ± 0.5° C) during repeated collections of sweat, or the bath temperature when the room temperature was kept the same (32 ± 0.3° C).

After collection, the sweat was filtered through Whatman No. 3 filter paper. Chloride concentrations were determined by a modification of the Volhard titration method (7); sodium, by the method of Butler and Tuthill (8) or by flame photometry with an internal standard; potassium, by the method of Fiske and Litarzek (9) or by flame photometry with an internal standard.

RESULTS

I. *Relations between sweat chloride concentration, intensity of thermal stimulation, skin temperature and rate of sweating.*

Data concerning these relations are contained in Table I, Experiments 1, 2, 3a, 4a. The subjects for these experiments were four normal adult males. Five to seven samples of sweat were collected from each subject. Collections were usually made at daily intervals.

The data demonstrate that there were great differences in the concentration of chloride in the several samples of sweat collected from each subject. For example, in the case of subject 3 the minimum concentration was 6 mEq per L. and the maximum, 54 mEq per L. Coefficients of correlation (Table II) indicate that variations in

TABLE I

Data illustrating the influence of certain non-hormonal and hormonal factors on sweat electrolyte concentrations

Exp. no.	Subj. no.	Sex and age	Diagnosis	Date	NaCl intake	Treatment	Room temp.	Bath temp.	Skin temp.	Rate of sweating	Sweat electrolytes (*mEq per L.*)			Comments
											Cl	Na	K	
					gms./m²/day		°C	°C	°C	*ml.**				
1	1	♂25	Normal	5/16	5	None	32.0	45.8	36.7	35	39	47		
				5/17	5	None	31.7	41.5	36.3	12	16	21	8.7	
				5/18	5	None	32.2	43.6	36.8	32	25	32	6.6	
				5/19	5	None	32.0	39.7	36.3	11	7	11	8.6	
				5/20	5	None	32.0	40.0	36.3	9	7	11		
2	2	♂27	Normal	6/6	5	None	28.2	44.4	35.6	20	20	31	8.2	
				6/7	5	None	33.2	44.6	36.6	44	42	54	7.0	
				6/8	5	None	26.6	44.4	34.9	10	8	16	11.1	
				6/9	5	None	33.7	45.0	36.7	47	39	54	7.6	
				6/10	5	None	27.5	44.1	36.0	18	12	19	8.3	
				6/11	5	None	35.5	44.5	37.5	54	43	60	7.1	
3a	3	♂27	Normal	7/7	5	None	29.7	44.7	36.7	18	16	22	8.5	
				7/8	5	None	31.8	44.3	36.8	33	24	28	7.0	
				7/9	5	None	25.9	44.6	36.5	20	17	23	7.5	
				7/10	5	None	37.3	44.5	37.7	71	54	62	6.4	
				7/11	5	None	26.8	40.6	36.0	7	6	10	8.5	
				7/12	5	None	33.8	44.7	37.5	55	35	39	6.5	
				7/13	5	D.C.A. 5 mgm. i.m. b.i.d.	28.1	44.6	37.0	37	18	22	6.3	D.C.A. started after sweat collection
3b	3			7/14	5	D.C.A. 5 mgm. i.m. b.i.d.	29.8	44.4	37.6	44	12	16	7.2	
				7/15	5	D.C.A. 5 mgm. i.m. b.i.d.	38.3	44.6	37.8	49	25	32	6.8	
				7/16	5	D.C.A. 5 mgm. i.m. b.i.d.	25.1	44.5	35.9	10	4	8	8.7	
				7/17	5	D.C.A. 5 mgm. i.m. b.i.d.	33.4	44.7	37.3	62	20	25	6.7	
				7/18	5	D.C.A. 5 mgm. i.m.	29.5	44.5	36.3	42	9	9	5.9	
4a	4	♂26	Normal	9/12	5	None	29.8	44.4	36.6	15	21	28	7.3	
				9/13	5	None	34.3	44.5	37.0	26	29	36	5.8	
				9/14	5	None	26.2	44.4	36.6	14	14	19	6.6	
				9/15	5	None	33.5	44.4	37.4	38	36	45	5.5	
				9/16	5	None	25.8	42.6	35.1	9	11	15	7.7	
				9/17	5	None								
				9/18	5	None								
				9/19	5	None	38.2	45.0	37.9	52	48	57	6.6	
				9/20	5	Progesterone 50 mgm. i.m.	32.7	44.3	36.1	23	20			Progesterone started after sweat collection
4b	4			9/21	5	Progesterone 50 mgm. i.m. b.i.d.	29.6	44.6	35.3	21	15			
				9/22	5	None								
				9/23	5	Progesterone 50 mgm. i.m. b.i.d.	25.9	42.8	35.6	13	11			
				9/24	5	Progesterone 50 mgm. i.m. b.i.d.	34.0	44.5	37.1	37	36			
				9/25	5	Progesterone 100 mgm. i.m.								
				9/26	5	None	30.1	44.5	37.0	32	25			

TABLE I—*Continued*

Exp. no.	Subj. no.	Sex and age	Diagnosis	Date	NaCl intake	Treatment	Room temp.	Bath temp.	Skin temp.	Rate of sweating	Sweat electrolytes (*mEq per L.*)			Comments
											Cl	Na	K	
					gms./m²/day		°C	°C	°C	*ml.**				
5a	5	♂17	Normal	8/21	5	None	35.1	44.5		14	8	13	9.1	
				8/22	5	None	35.5	44.5		29	12	19	5.5	
				8/23	5	ACTH 25 mgm. b.i.d.	35.3	44.1		34	16	22	4.4	ACTH started after sweat collection
5b	5			8/24	5	ACTH 25 mgm. q.i.d.	35.9	44.4		55	18	27	5.2	
				8/25	5	ACTH 25 mgm. q.i.d.	36.0	44.4		53	12	11	5.1	
				8/26	5	ACTH b.i.d.	36.0	44.3		53	10	13	5.7	
5c	5			8/27	5	None	36.0	44.3		54	11	16	5.4	
				8/28	5	None	36.0	44.4		55	16	19	5.0	
				8/29	5	None	36.1	44.4		54	18	21	5.4	
6a	6	♂24	Normal	10/13	5	None	29.0	44.2		17	12	22	8	
				10/14	5	None	32.7	44.3		29	19	23	5.5	
				10/15	5	Methyl testosterone 25 mgm. q.i.d. p.o.	36.2	44.4		32	23	27	5.1	Testosterone started after sweat collection
				10/18	5	Methyl testosterone 25 mgm. q.i.d. p.o.				20	18	26	6.1	Treatment with methyl testosterone since 10/15
				10/19	5	Methyl testosterone 25 mgm. q.i.d. p.o.				12	13	19	7.1	
				10/20	5	None				37	34		10.1	
7	6	♂24	Normal	3/10	5	None	30.5	44.4		7	17	23	11.6	
				3/11	5	None	35.7	44.7		17	28	30	7.1	
				3/12	5	None	36.6	44.4		18	19	23	5.6	
8a	7	♀25	Normal	7/24	5	None	37.1	45.2		33	47	58	5.5	
				7/25	5	None	36.9	44.7		33	46	56	4.6	
				7/26	5	None	36.4	44.4		28	38	47	5.5	
8b	7			7/27	5	Estradiol benz. 5 mgm. i.m.	36.4	44.1		20	26	33	5.7	
				7/28	5	Estradiol benz. 5 mgm. i.m.	35.9	44.7		27	29	36	5.1	
				7/29	5	Estradiol benz. 5 mgm. i.m.	36.4	44.5		34	34	42	5.2	
9	7	♀25	Normal	12/10	5	None	37.3	44.2		17	56	64	7.3	
				12/11	5	None	37.0	44.0		18	58	65	7.5	
				12/12	5	None	36.3	43.9		18	49	60	7.4	
10a	8	♂34	Normal	4/29	5	None	33.4	44.7		48	22	29	5.4	Subject frequently exposed to high temperatures prior to acting as experimental subject
				4/30	5	None								
				5/1	5	None	33.2	44.6		41	20	26	5.2	
				5/2	5	None	32.4	44.0		43	15	22	5.4	
				5/3	5	None	32.8	44.2		36	13	18	5.0	
				5/4	4	Cortisone 100 mgm. i.m.	32.7	44.3		36	14	16	4.5	
10b	8			5/5	5	Cortisone 100 mgm. i.m. b.i.d.	33.3	44.3		44	16	21	4.5	
				5/6	5	Cortisone 100 mgm. i.m. b.i.d.	33.2	43.4		51	19	20	4.5	
				5/7	5	Cortisone 100 mgm. i.m.	34.3	43.7		41	19	23	5.2	
10c	8			5/8	5	None	32.7	44.2		35	14	16	4.8	
				5/9	5	None	33.1	44.0		42	13	15	4.7	
				5/10	5	None	33.2	44.2		58	24	29	4.8	
				5/11	5	None	33.3	44.4		43	19	22	4.8	
				5/12	5	None								
				5/13	5	None	32.7	44.0		35	14	17	5.3	
				5/14	5	None				41	13	18	4.3	
				5/15	5	None								
				5/16	5	None	32.9	44.1		44	16	22	4.8	
10d	8			5/17	10	None								
				5/18	15	None	33.0	44.2		39	16	21	5.1	
				5/19	15	None	33.0	44.0		35	20	26	5.0	
				5/20	15	None	33.5	44.4		42	32	39	4.3	
				5/21	15	None								
				5/22	15	None	33.5	44.4		42	30	36	4.2	
				5/23	15	None	33.7	44.2		48	37	43	4.0	

TABLE I—*Continued*

Exp. no.	Subj. no.	Sex and age	Diagnosis	Date	NaCl intake	Treatment	Room temp.	Bath temp.	Skin temp.	Rate of sweating	Sweat electrolytes (*mEq per L.*)			Comments
											Cl	Na	K	
					gms./m²/day		°C	°C	°C	*ml.**				
11a	8	♂33	Normal	12/13	5	None				20	21	27	6.2	Subject frequently exposed to high temperatures prior to acting as experimental subject
				12/14	5	None				29	22	28	6.3	
				12/15	5	None	30.1	44.8		17	11	15	5.2	
11b	8			12/16	5	K supplement								
				12/17	5	K supplement	33.5	43.8		22	13	17	9.1	27 gms. K citrate, .745 gms. K_2HPO_4, 3.78 gms. KH_2PO_4, added to daily diet
				12/18	5	K supplement	30.4	45.3		27	14	17	7.6	
				12/19	5	K supplement	28.8	44.7		25	14	15	7.3	
				12/20	5	K supplement	28.7	44.7		21	11	13	6.4	
				12/21	5	K supplement	28.3	44.5		19	10	12	6.4	
11c	8			12/22	5	None								
				12/23	5	None								
				12/24	5	None	29.9	44		12	10	13	7.3	
				12/25	5	None	29.4	44.4		26	17	21	7.0	
				12/26	5	None								
				12/27	5	None	28.1	45.5		24	19	16	3.6	
				12/28	5	None	33.7	44.9		27	21	18	3.4	
				12/29	5	None	24.5	45.0		11	8	14	7.9	
12	9	♂24	Normal	5/25	15	None	39.6	44.4		60	79	96	6	Legs immersed in water bath
				5/25		None	41.1	44.3		44	72	83	5.2	
				5/25		None	34.9	44.0		40	67	78	5.0	
				5/25		None	31.2	41.2		15	43	54	5.5	
				5/25		None	31.0	42.9		24	50	61	5.4	
13	10	♂24	Normal	6/21	24	None	41.7	44.0		40	87	98	4.8	Legs immersed in water bath
				6/21		None	37.5	44.8		17	75	88	4.2	
				6/21		None	36.5	41.5		9	52	63	5.6	
				6/21		None	36.8	42.1		25	74	81	4.2	
				6/21		None	36.8	40.3		15	66	76	4.0	
14a	11	♂50	Addison's disease	8/3	2	D.C.A. pellets	25.0	44.1		16	20	28	6.8	
				8/4	2	D.C.A. pellets	28.1	44.4	34.8	14	19	26	7.4	
				8/5	7	D.C.A. pellets	33.6	44.3	36.6	18	26	31	6.4	
14b	11			8/9	7	D.C.A. pellets	27.2	44.5	36.4	8	17	18	5.6	Daily intake NaCl 7 gms./m²/day since 8/5
				8/10	7	D.C.A. pellets	32.7	44.4	37.1	18	27	36	7.1	
				8/11	7	D.C.A. pellets				22	34	44	7.2	
15	12	♂47	Addison's disease	5/11	4–6	None	34.2			3.1	37			Arm not immersed in water bath
				5/11		None	37.3	44.2		32	88	102	6	
				5/12		None	30.3	44.1		15	62			
16	13	♂48	Essential hypertension	2/24	5	None	37.2	44.3		21	68	81	6.4	Daily intake NaCl 5 gms./m²/day since 2/15
				2/25	5	None	30.8	44.5		17	48		7.6	
				2/26	5	None	32.8	44.4		19	53	67	6.6	
17	14	♂53	Essential hypertension	3/27	5	None	31.2	44.4		13	5	11	7.0	Daily intake NaCl 5 gms./m²/day since 3/18/50
				3/28	5	None	34.7	44.3		19	7	13	6.1	
				3/29	5	None	36.7	44.2		26	10	16	6.3	

* Ml. per hour per 0.1 m² of skin surface.

TABLE II

*Coefficients of correlation**

Factors correlated	Exp. no. (Table I)			
	1	2	3a	4a
Rate of sweating vs. sweat chloride concentration	+.933	+.979	+.963	+.972
Intensity of stimulation vs. sweat chloride concentration	+.996	+.978	+.923	+.901
Skin temperature vs. sweat chloride concentration	*+.829*	+.890	+.919	+.892
Rate of sweating vs. intensity of stimulation	+.998	+.998	+.879	+.887
Rate of sweating vs. skin temperature	+.969	+.947	+.971	+.861
Intensity of stimulation vs. skin temperature	*+.860*	+.936	+.886	*+.678*

* All coefficients are statistically significant ($p < 0.05$) except those italicized.

sweat chloride concentration were in each subject significantly correlated with *a*) rate of sweating, *b*) skin temperature and *c*) intensity of the thermal stimulus. In addition, they show that rate of sweating, skin temperature and the intensity of the thermal stimulus were significantly correlated with each other in almost all instances.

These findings point to a presumably non-hormonal factor capable of profoundly modifying the concentration of chloride in sweat obtained from normal subjects receiving a constant intake of sodium chloride. Consequently, it would appear that the intensity of action of the factor should be taken into consideration if sweat electrolyte composition is to be used as an index of an individual's hormonal status. Consideration of the data suggests that this may be done reasonably satisfactorily by measuring the rate of sweating. Accordingly, there follows a more detailed consideration of the relation between sweat chloride concentration and rate of sweating.

II. *Detailed study of the relation between sweat chloride concentration and rate of sweating.*

In all experiments summarized in Table I, data concerning this relation are recorded. Representative data drawn from this table (Experiments 3a, 3b, 13, 14b, 15) are presented graphically in Figure 1.

The experiments summarized in the graph were performed on *a*) a normal adult male, maintained on the standard diet, both before (Curve A) and

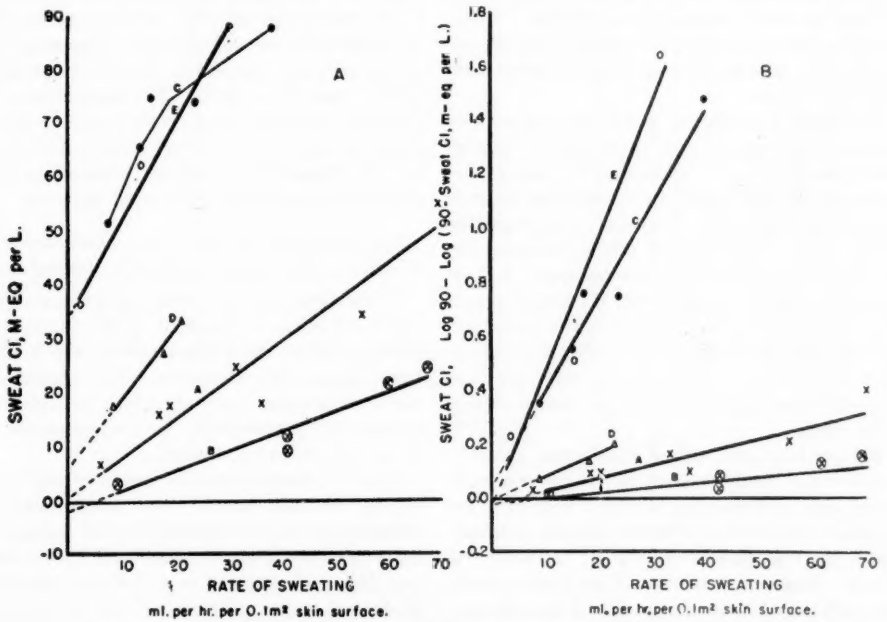
SWEAT Cl, M-EQ per L.
A
B
SWEAT Cl, Log 90 - Log (90 - Sweat Cl, m-eq per L.)
RATE OF SWEATING
ml. per hr. per 0.1m² skin surface.
RATE OF SWEATING
ml. per hr. per 0.1m² skin surface.

FIG. 1. RELATION BETWEEN SWEAT CHLORIDE CONCENTRATION AND RATE OF SWEATING

Data from Table I: Curve A—Experiment 3a; Curve B—Experiment 3b; Curve C—Experiment 13; Curve D—Experiment 14b; Curve E—Experiment 15. In both sections of the figure (A and B), absolute values for rate of sweating are indicated by the scale along the abscissa. In the left hand Section A, absolute chloride values are given according to the scale along the left hand ordinate. In the right hand Section B, the values indicated along the ordinate scale were found by subtracting from log 90 the log of (90 minus sweat chloride in mEq per L.). The heavy solid lines were calculated by the "method of least squares." The light solid line (Curve C, Section A) was drawn free-hand. Interrupted lines represent extrapolations of the heavy solid lines to the point where they would theoretically intersect the ordinate. The slopes of the solid lines of Section B define the value for the sweat chloride-rate index.

while he was being given desoxycorticosterone acetate (D.C.A.), 9 mgm. b.i.d., i.m. (Curve B); *b*) a normal male maintained on a diet which provided him with 24 gms. of sodium chloride per m^2 per day (Curve C); *c*) an Addisonian adult male whose diet contained 6.5 gms. of sodium chloride per m^2 per day and whose symptoms were well controlled by recently implanted pellets of D.C.A. (Curve D); and *d*) an Addisonian adult male whose diet contained approximately 5 gms. of sodium chloride per m^2 per day and whose symptoms were poorly controlled by 3 D.C.A. pellets implanted eight months previously (Curve E).

Figure 1 consists of two regression graphs, A and B. The same data are used in each and in both the abscissa is the rate of sweating and the ordinate is sweat chloride concentration. However, for reasons which will shortly become apparent, the methods for expressing chloride concentration differ.

In Figure 1-A chloride concentrations are expressed simply as mEq per L. of sweat. Note in this figure that in all instances there was an increase in the concentration of chloride as the rate of sweating increased. However, the properties of the curves describing the relation between the two variables appear to be very different. In four instances (Curves A, B, D and E) linear regression lines describe the relation satisfactorily but on extension they intercept the ordinate at widely different points (-1.8 to $+33.2$ mEq per L.). In the fifth instance (Curve C), the relation clearly is not linear.

Figure 1-A also shows that sweat chloride concentration measurements do not *per se* always permit one to distinguish between sweat from individuals subjected to different degrees of stimulation by the adrenal cortex or administered D.C.A. However, inspection of the figure reveals that such a distinction can be made if chloride concentration is viewed with relation to rate of sweating. Although evident on inspection, differences between the curves of Figure 1-A cannot be described and quantitated satisfactorily in simple arithmetic terms.

Because it seemed highly desirable to have a generally applicable, simple arithmetic method describing the relation between chloride concentration and rate of sweating, other methods of expressing the data were sought. Examination of available data suggested that the relation between sweat chloride concentration and rate of sweating might be described satisfactorily by a series of catalytic curves asymptotic at a chloride concentration of approximately 90 mEq per L. Accordingly, to test this possibility values for sweat chloride concentration were expressed as log 90 minus log (90 minus sweat chloride concentration in mEq per L.). The resultant values have been plotted (ordinate) against rate of sweating (abscissa) in Figure 1-B. Note that in this graph all curves are linear and all, on extension, intercept the ordinate very close to the origin. Differences between them consist only in differences in slope. This fact permits the relation between sweat chloride concentration and rate of sweating to be represented with fair accuracy by dividing the ordinate by the abscissa value. The magnitude of the resultant quotient is directly proportional to the slope of the curve. For convenience, this quotient, expressed as a simple number, will be called the *sweat chloride-rate index*. This index is used henceforth to indicate relations between chloride concentration and rate of sweating.

III. *Relations between the concentration of chloride, sodium, and potassium in thermal sweat.*

In the preceding and following sections of this paper, attention is restricted to the response of chloride ion to the various stimuli which affect sweat electrolyte composition. The present section indicates the extent to which chloride concentration measurements are representative of sweat sodium and potassium.

Figure 2 demonstrates by means of regression graphs relations between the concentration of chloride and *a*) the concentration of sodium, *b*) the concentration of potassium and *c*) the ratio, $(a)/(b)$, as determined in 57 different specimens of thermal sweat. Fifty-one of the 57 specimens were collected from normal subjects. Of these 35 came from subjects eating the standard diet. Twenty-three of the 35 specimens were collected from subjects who were not receiving hormonal treatment; the remaining 12 specimens were collected from subjects being treated with D.C.A. Seven specimens were collected from a subject whose daily diet contained a great excess of sodium over chloride (220 mEq Na, 12 mEq Cl)

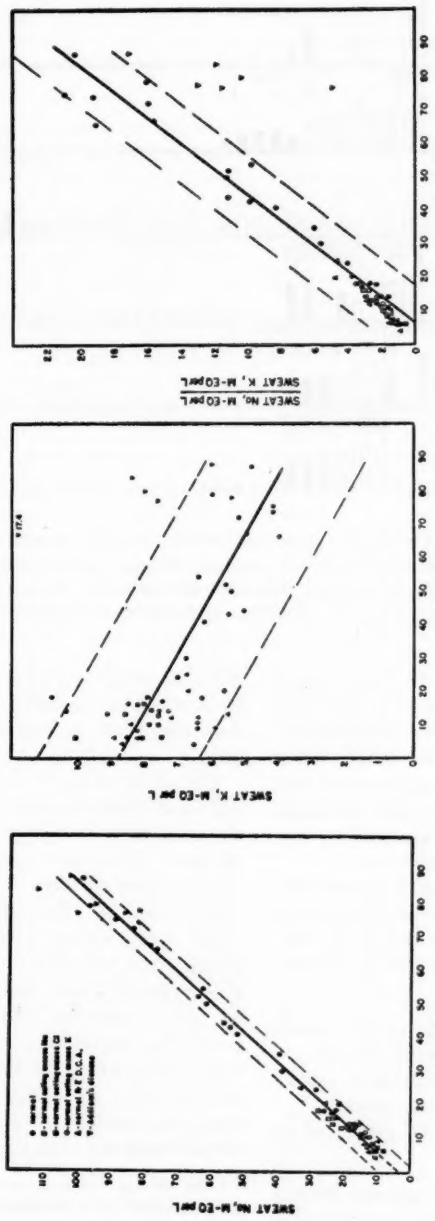

FIG. 2. RELATIONS BETWEEN THE CONCENTRATIONS OF CHLORIDE, SODIUM, AND POTASSIUM IN THERMAL SWEAT

The broken lines indicate limits of normal variability ($y \pm$ standard error of estimate).

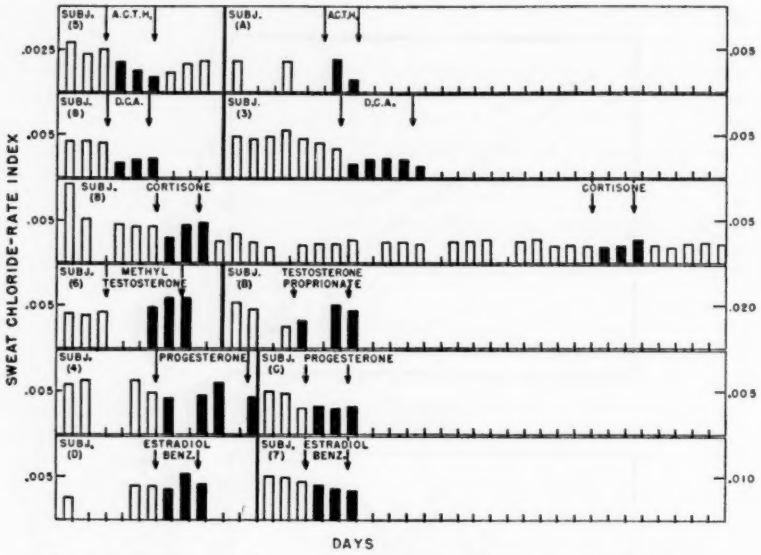

FIG. 3. THE EFFECT OF CERTAIN HORMONES ON THE SWEAT CHLORIDE-RATE INDEX

ACTH 25 mgm. q.i.d.; D.C.A. 5 mgm. b.i.d.; Cortisone 75 mgm. and 100 mgm. b.i.d.; Methyl testosterone 25 mgm. q.i.d.; Testosterone propionate 100 mgm. q.d.; Progesterone 50 mgm. b.i.d.; Estradiol benzoate 5 mgm. q.d. Subjects are designated by number if data are included in Table I.

and four were collected from the same subject when his daily diet contained a great excess of chloride over sodium (15 mEq Na, 220 mEq Cl). Five specimens were obtained from a subject whose basal (standard) daily diet was supplemented with large amounts of potassium salts (370 mEq K). Six specimens were collected from patients having poorly controlled Addison's disease.

In the sweats from the untreated normal subjects eating the standard diet, the relation between the concentration of chloride and each of the other three variables is linear. These relations, expressed mathematically were:

a) [Na] = 1.12 [Cl] + 3 mEq per L. (standard error of estimate 2.6 mEq per L.)
b) [K] = 0.056 [Cl] + 8.8 mEq per L. (standard error of estimate 1.2 mEq per L.)
c) [Na]/[K] = 0.26 [Cl] − 1.7 (standard error of estimate, 1.52).

The coefficients of correlation between chloride concentration and each of the other three variables are all significant. As the concentration of chloride rises the concentration of sodium and the ratio between the concentrations of sodium and potassium rise while the concentration of potassium falls.

The values for the 11 samples of sweat from the individual eating the diets grossly abnormal with respect to their electrolyte contents all fell within the range of normal variability.[3] In the 12 samples of sweat collected from individuals treated with D.C.A. all values fell within the "normal range" except for one value for potassium concentration which fell below the normal range. In the six samples of sweat obtained from patients having poorly controlled Addison's disease, in relation to the concentration of chloride, *a*) three sodium concentrations fell within, two fell above and one fell below the normal range, *b*) two potassium concentrations fell within and four above the normal range and *c*) all six ratios between the con-

[3] The normal range of variability was taken to be $y \pm 2$ standard error of estimate where y is [Na], [K] or [Na]/[K]. Such a range includes 96 per cent of determinations.

centrations of sodium and potassium were below the normal range.

IV. *Relations between sweat chloride index and hormonal status.*

A. *The effect of certain hormones on the sweat chloride-rate index.* In this series of experiments one subject, who received ACTH, had rheumatoid arthritis. All other subjects were normal. Cortisone acetate was given to one subject on two occasions. Methyl testosterone and testosterone propionate were each given to one subject. The other hormones were each given to two subjects. Methyl testosterone was taken by mouth. The other hormones were administered intramuscularly. The hormones and dosages employed were as follows: Adrenocorticotropic hormone (Armour Standard ACTH) 25 mgm. b.i.d. or q.i.d. (see Table I); desoxycorticosterone acetate (D.C.A.) (Percorten, Ciba) 5 mgm. b.i.d.; cortisone acetate (Cortone, Merck) 75 mgm. b.i.d. on one occasion and 100 mgm. b.i.d. on another; methyl testosterone (Oreton-M, Schering) 25 mgm. q.i.d.; testosterone propionate (Oreton, Schering) 100 mgm. daily; progesterone (Proluton, Schering)50 mgm. b.i.d.; estradiol benzoate (Progynon-B, Schering) 5 mgm. daily. In most instances the hormones were administered over a three to five day period.

Figure 3 represents the results of these experiments. The administration of either ACTH or D.C.A. was accompanied by striking reductions in the value of the index in all instances. The administration of methyl testosterone was accompanied by a significant rise in index values. While cortisone, progesterone and testosterone propionate had no statistically significant effect on the sweat chloride-rate index, inspection of the data suggests that their administration was associated with a slight rise in index values. It is possible that longer administration of cortisone would have resulted in a tendency to lowered index values. In one instance the administration of estradiol benzoate was accompanied by a significant reduction in the index; in the other instance this hormone had if anything an opposite effect.

B. *A comparison of the sweat chloride-rate index in normal and hypoadrenocortical subjects.* Figure 4 presents measurements of the sweat chloride-rate index obtained as a result of the first sweating in the cases of *a*) 10 normal subjects (seven male, three female) eating the standard diet during the summer; *b*) nine normal subjects (five male, four female) eating the standard diet during the winter; *c*) seven hypoadrenocortical subjects of which three were male and four female. Collections of sweat were made from the hypoadrenocortical patients during both seasons of the year. One of these patients ate the standard diet. The others were on hospital diets estimated to contain 4–6 gms. of sodium chloride per m^2 per day.

In the normal subjects eating the standard diet, the average value for the index was during the summer .0054 (range .0029 to .0097) and during the winter .0165 (range .0111 to .0249). In the hypoadrenocortical subjects the average was .1095 (range .0348 to .1809). In none of the three groups did the sex of the subject appear to affect the index value.

V. *The sweat chloride-rate index in studies of adrenal function in man.*

A. *Effect of varying the dietary intake of sodium chloride and potassium salts.* Figure 5 portrays data obtained in experiments involving one normal male (Table I, Experiments No. 10c-d), one diabetic female and one patient having Addison's disease (Table I, Experiments No. 14a-b). Their dietary intakes of sodium chloride per day varied

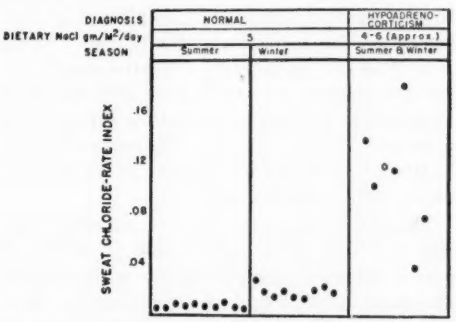

FIG. 4. SWEAT CHLORIDE-RATE INDICES FOR THE FIRST SWEATING OF NORMAL AND HYPOADRENOCORTICAL SUBJECTS

In the hypoadrenocortical group, black circles represent patients with primary adrenal cortical insufficiency and the single white circle represents a patient with hypoadrenocorticism secondary to hypopituitarism.

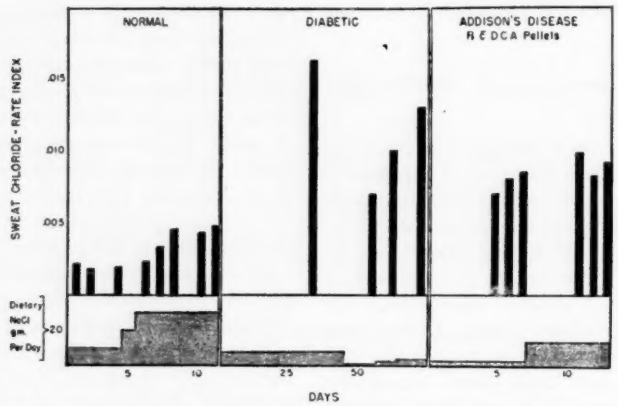

FIG. 5. THE EFFECT ON THE SWEAT CHLORIDE-RATE INDEX OF VARYING DIETARY SODIUM CHLORIDE

Differences noted in the normal and diabetic subjects following changes in dietary NaCl intake are statistically significant; the difference between the two groups of values obtained in the Addisonian patient is not statistically significant.

between 10 and 30 gms., 0.6 and 8.6 gms. and 3.5 and 11.5 gms., respectively. Note that the sweat chloride-rate index closely parallels the sodium chloride intake in the normal and in the diabetic subjects. In the Addisonian patient, no statistically significant change in the index value occurred.

In Experiments 12 and 13, Table I, two normal male subjects were given diets containing very large amounts of sodium chloride (15 and 24 gms. per m^2 per day, respectively) for 10 days. At the time of the first sweating the respective sweat chloride-rate indices were .0152 and .0369, the latter being within the range observed for patients with untreated Addison's disease. The range of indices in 10 comparable normal subjects on the standard diet is .0029 to .0097 (Figure 4).

Figure 6 describes the effect on the sweat chloride-rate index of a normal male of adding 27 gms. of potassium citrate, 8.02 gms. of dipotassium phosphate and 3.78 gms. of monopotassium phosphate (370 mEq of K) to a standard diet. Note that the index value was significantly lower during the high potassium period than during two control periods.

B. *Effects of exposure to heat.* Figure 7 describes the effect on the sweat chloride-rate index of normal subjects of *a*) season (see also Figure 4) and *b*) repeated sweating. It is evident that the indices are considerably lower during the "summer" (May 1 to October 31) than during the "winter" (November 1 to April 30). In the winter, but not in the summer, there is a well defined tendency for index values to fall when the subject

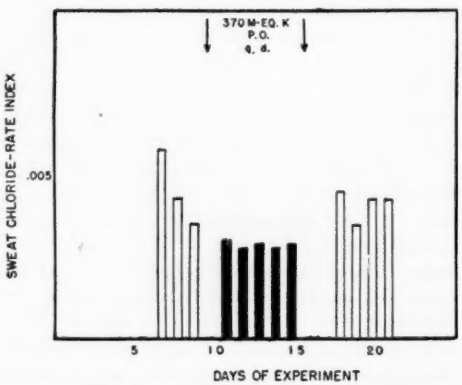

FIG. 6. THE EFFECT ON THE SWEAT CHLORIDE-RATE INDEX OF INCREASING THE DIETARY INTAKE OF POTASSIUM SALTS

The values during the high potassium period are significantly different from the control values.

is sweated repeatedly on consecutive days. The observations of Figure 7 plus other incomplete data suggest that between three and approximately eight consecutive days of hourly exposure to intense heat are required to induce a drop in index values from average winter to average summer levels.

C. *Hypertension.* Figure 8 shows the sweat chloride-rate indices of four patients suffering idiopathic hypertension. These measurements were carried out during the winter. The patients had been maintained on diets low in sodium (estimated to contain about 1 gm. of sodium chloride per m^2 per day) for from five to 20 months prior to the beginning of the experiments. For the first 10 days of the experiment, during which no sweat was collected, and for the subsequent two to three days during which collections were made at daily intervals, they were maintained on the standard diet.

It is seen that sweat chloride-rate indices for two of the patients fell within the normal range (Figure 7); in the other two patients the values definitely were subnormal.

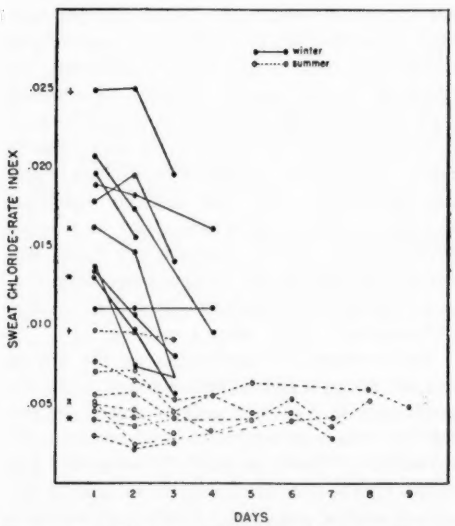

FIG. 7. THE EFFECT OF SEASON AND REPEATED SWEATINGS ON THE SWEAT CHLORIDE-RATE INDEX

At the left of some of the curves are symbols. Each type of symbol represents a single individual. Thus repeat studies on the same individual can be recognized by symbol duplication.

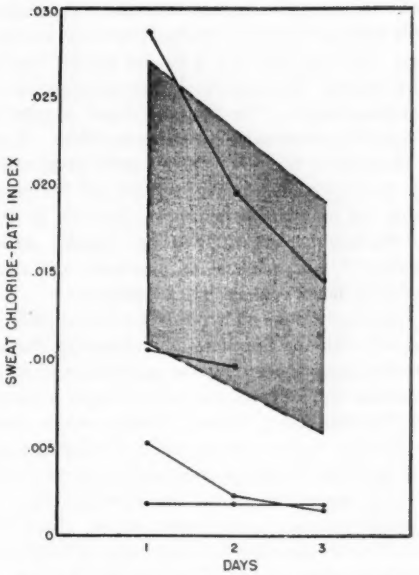

FIG. 8. VALUES FOR THE SWEAT CHLORIDE-RATE INDEX OF FOUR HYPERTENSIVE SUBJECTS

These values were obtained during the winter. The shaded area gives the range of values obtained on normal subjects during the same season as indicated in Figure 7.

DISCUSSION

The initial studies described in this report show clearly that sweat electrolyte composition is influenced almost as much by non-endocrine factors related to intensity of thermal stimulus, skin temperature and rate of sweating as by active adrenal cortical hormones. Under conditions of constant hormonal status, sweat electrolyte concentrations bear a mathematically predictable relation to these non-endocrine variables. A change in intensity of action of hormones which influence sweat formation results in an alteration in sweat electrolyte composition relative to thermal stimulus, skin temperature and rate of sweating.

The sweat chloride-rate index was evolved in an effort to express such changes in sweat electrolytes relative to these non-hormonal factors in a practically useful manner. Values for the concentration of chloride bear a closely predictable direct relation to those of sodium and an approximate, inverse relation to those of potassium in

sweat. Hence chloride may be considered satisfactorily representative of the other two sweat electrolytes. Rate of sweating does not always bear a close relation to intensity of thermal stimulus or skin temperature. On the other hand, it usually is roughly proportional to these variables. Rate of sweating is closely correlated with sweat chloride concentration when the dietary and hormonal status are maintained constant. Because of this and the fact that rate of sweating is readily measureable, it was selected to represent the non-endocrine factors in the index equation.

The observations on hypoadrenocortical patients coupled with the hormone administration studies provide clues concerning the probable nature of endocrine factors responsible for changes in sweat chloride-rate index values. Index values were consistently higher than normal in hypoadrenocortical patients. Desoxycorticosterone acetate was the only one of the steroid hormones to induce a marked decrease in the index value. This occurred within one day. Interestingly, 17-hydroxy-11-dehydrocorticosterone (cortisone) induced if anything a slight rise in index values over a period of three days.

These data suggest that sweat chloride-rate index values bear an inverse relation to the concentration of adrenal cortical hormones which, like desoxycorticosterone, act chiefly upon body electrolyte and water metabolism (10). Adrenocorticotropic hormone induced as great and as rapid a fall in the index value of normal subjects as did desoxycorticosterone acetate. It therefore appears that the human adrenal cortex secretes a hormone with desoxycorticosterone-like properties in response to adrenocorticotropin stimulation.

While the estrogens and androgens given were not wholly without effect upon sweat chloride-rate index values, they did not appear to exert a striking and consistent regulatory influence. This suggests that their influence upon sweat formation may have been accomplished indirectly by stimulating or inhibiting pituitary adrenocorticotropic hormone production rather than by direct action upon the sweat glands. Inhibition of adrenocorticotropic hormone secretion and hence of adrenal cortical activity might account for the slight rise in index values observed following cortisone, progesterone and testosterone. This thesis is compatible with the fact that cortisone and methyl testosterone act to suppress adrenal cortical urinary 17-ketosteroid precursor production, presumably by inhibiting adrenocorticotropin secretion (11, 12). Methyl testosterone also can inhibit 11-17-oxycorticosteroid excretion (13). The single observation that estradiol may induce a lowering of index values likewise is in keeping with the fact that estrogens can cause adrenal cortical hyperplasia by stimulating secretion of adrenocorticotropic hormone (14).

The foregoing considerations lead to the conclusion that sweat chloride-rate index values are determined chiefly by adrenal cortical hormones of the 11-desoxycorticosterone type. A rise in index values therefore suggests a decrease, and a fall in index values an increase in adrenal cortical activity.

The present studies of adrenal cortical status based on measurements of this index extend and confirm observations of other workers which suggest that the adrenal cortices undergo physiologic alterations in activity in response to certain situations (14–16). Thus the consistent tendency for the sweat chloride-rate index to fall when normal persons are subjected to low sodium chloride diets, high potassium diets or repeated exposure to heat indicate that these factors induce a physiologic increase in adrenal cortical activity. By contrast, high sodium chloride diets prompt the index values of normal persons to rise to levels comparable to those observed in patients with primary hypoadrenocorticism. The fact that no significant changes in index values took place in a hypoadrenocortical patient when the salt intake was varied indicates that the changes observed in normal subjects were dependent upon the presence of functionally intact adrenal cortices.

The comparative observations on the normal subjects taking a high sodium diet and on the hypoadrenocortical patients indicate that under certain circumstances absolute rates of adrenal cortical secretory activity attained physiologically may closely approximate those observed in patients with adrenal cortical pathology. Similar information is provided by studies of urinary 11-17-oxycorticosteroid excretion in health and disease (17). In other words, pathologic is distinguished from physiologic adrenal cortical function by the relation of secretory activity to physiologic, homeo-

static needs rather than by absolute differences in secretory rate.

Preliminary observations on patients with idiopathic hypertension indicate that the sweat chloride-rate index may provide interesting diagnostic information in certain presently obscure diseases. Two of the four patients studied had values suggestive of pathologic hyperproduction of the electrolyte-water regulating type of adrenal cortical hormone.

SUMMARY AND CONCLUSIONS

1. Intensity of thermal stimulus, skin temperature and rate of sweating bear an important relation to sweat electrolyte composition. Certain hormones act to modify sweat composition relative to these factors. Such hormonal action can be appraised by use of an index which expresses sweat chloride concentration values relative to rate of sweating.

2. Studies suggest that the chief determinant of sweat chloride-rate index values is an adrenal cortical hormone which, like desoxycorticosterone, acts chiefly upon electrolyte and water metabolism.

3. Measurements of the sweat chloride-rate index suggest strongly that the normal adrenal cortices can undergo wide variations in activity in response to physiologic, homeostatic needs. Under physiologic conditions, adrenal cortical production of desoxycorticosterone-like hormones may subside to levels comparable to those existing in patients with primary pathologic hypoadrenocorticism.

4. The possibility that the sweat chloride-rate index measurement may have diagnostic value in miscellaneous conditions is exemplified by preliminary observations on four patients with idiopathic hypertension.

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THE EFFECT OF LIPIODOL ON ALVEOLAR GAS EXCHANGE [1, 2]

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Within the past 15 years the advances in intrathoracic surgery have made visualization of the bronchial tree with contrast media, particularly iodized poppyseed oil (lipiodol), a common practice. Despite the established safety of the procedure in the vast majority of instances, repeated questions have been raised as to the innocuousness of the oil in the pulmonary parenchyma. The feeling has been expressed by some authors that it is unwise to perform any intrathoracic procedure for several weeks to several months following the administration of lipiodol. Thus Churchill (1) has advised delaying operation for a minimum of six weeks after bronchography because of fear of postoperative atelectasis and pneumonitis due to the oil. Belsey (2) was of the same opinion and a more recent paper by Miscall (3) indicates a persistence of this feeling.

Pinkerton (4) has concluded from animal experiments that lipiodol in the absence of infection produces almost no reaction in the lungs. In clinical use, however, some evidence of lipoid granuloma has been observed (5).

It is also conceivable that lipiodol in the alveoli might impair transalveolar gas exchange and thus interfere with recovery from an intrathoracic operation. Whereas immediately after introduction the material is limited chiefly to the bronchial tree, soon thereafter its presence can be demonstrated diffusely filling the lung parenchyma where it may persist for several weeks or longer. In an attempt to evaluate the possibility of deficiencies of transalveolar diffusion following the administration of lipiodol the present study was undertaken.

MATERIAL AND METHODS

Ten patients who were to have lipiodol bronchograms were studied before, within several hours after the instillation of the oil and on the following day. Bilateral bronchograms were performed by the aspiration technique using 10 cc. of oil on each side. Since there is considerable puddling in the pyriform sinuses less than this actually enters the lungs. Nevertheless, with this method satisfactory mapping of the entire bronchial tree is regularly obtained. It should be stressed that a significant amount of residual lipiodol was seen in the alveoli of all patients on fluoroscopy at the time the last tests were performed.

The techniques employed to study the patients were: *1*) determination of the oxygen pressure (P_{O_2}) gradient between the alveolar air ($P_{A_{O_2}}$) and the arterial blood ($P_{a_{O_2}}$) [4]; *2*) measurement of the arterial oxygen saturation; *3*) determination of the time required to attain full saturation when the subject was switched from room air to 100% oxygen and *4*) determination of vital capacity and its subdivisions.

The patient was brought to the laboratory without medication, but not necessarily in fasting condition. After resting 10–15 minutes on a stretcher in the supine position a sample of arterial blood was withdrawn from the femoral artery in the course of one to two minutes, during which period the expired air was collected in a Douglas bag, which previously had been washed out twice with the same gas. The patient was provided with a mouthpiece and nose clip and a system of valves with small dead space was used for the air collection.

Precautions were taken to disturb the patient as little as possible. Thorough novocaine infiltration preceded the arterial puncture, and several minutes were allowed to elapse between the arterial puncture and the collection of blood and expired air. Twenty cc. of blood were collected in a lightly oiled syringe containing 0.5 cc. of a mixture of eight parts of commercial heparin solution and two parts of a saturated solution of sodium fluoride.

The direct determination of oxygen and carbon dioxide tensions of this blood sample was then immediately performed by a technique which has been described in detail elsewhere (7). In principle, it consists of the equilibra-

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[4] The symbols used throughout this publication have been made to conform with those recommended at the April, 1950, meeting of the American Physiological Society (6). Substitutions for formerly used symbols are as follows: 'P' is gas pressure in general; 'F' is fractional concentration in dry gas phase; 'I' is inspired gas; 'A' is alveolar gas; 'a' is arterial.

tion of 17 cc. of blood with an 80 cu. mm. gas bubble (initial composition of the bubble 12.15% O_2, 5.25% CO_2) for 10 minutes at 37° C. At the end of this time pressure equilibration of the respiratory gases in blood and gas phases is obtained with less than 1 mm. Hg change in gas pressure in the liquid phase. The bubble is then transferred to a Scholander micro gas analyzer, where its composition is determined. On the assumption that the total gas pressure of the bubble is equal to atmospheric, the partial pressures in the bubble, and therefore, in the blood, are thus found. This assumption is justified in the case of normal arterial blood. Though in some of the patients studied the total arterial gas pressure was probably somewhat less than atmospheric, this would not affect the partial pressures of CO_2 and O_2 to a significant degree. The error of this method, as found by performing the above determination on blood of known gaseous composition, amounts to $+0.1 \pm 3.3$ mm. Hg for O_2 and -5.3 ± 2.4 mm. Hg for CO_2. A correction of $+3.0$ mm. Hg has been made for the CO_2 pressure due to the diluting effect of the anticoagulant.

The "ideal" alveolar oxygen pressure was determined from analysis of the expired air, collected simultaneously with the blood, and from the directly determined arterial CO_2 pressure. As defined by Riley and associates (8, 9) and Rahn (10) the "ideal" or "effective" alveolar pressures are those which if present continuously and uniformly in all functioning alveoli, and if complete equilibrium were reached between the blood and gas phases in the lung, would permit CO_2 and O_2 exchange in amounts equal to that measured from analysis of inspired and expired air. As alveolar ventilation-perfusion relationships vary in different parts of the normal, and particularly of the pathologic lung, and as equilibrium of oxygen between blood and alveolar air in the abnormal lung often is not accomplished, "ideal" alveolar air must remain a theoretical quantity. Its practical usefulness lies in the fact that reproducible values can be assigned to alveolar oxygen and carbon dioxide pressures, whereas this would be impossible to accomplish through direct sampling in the many instances of non-homogeneous alveolar air. The calculation of the "ideal" alveolar P_{O_2} is based on the assumptions that the alveolar respiratory quotient equals that of expired air, and that the alveolar CO_2 tension equals that of arterial blood. Whereas the former assumption seems to be correct (10), the latter may not be strictly true in cases of severe pulmonary disease. However, the patients studied in this paper probably did not have a significant arterial-alveolar P_{CO_2} gradient.

The equation used to calculate "effective" alveolar P_{O_2} was the following (9, 11):

$$P_{A_{O_2}} = P_{I_{O_2}} + \frac{P_{a_{O_2}} \times F_{I_{O_2}}(1 - RQ)}{100 \times RQ} - \frac{P_{a_{CO_2}}}{RQ}.$$

As various techniques are in use for the direct determination of blood gas tensions and for the determination of alveolar air composition, the oxygen gradient in 10 healthy male subjects from 25–35 years of age was determined, using the technique described above. The results are summarized in Table I. The average gradient was 3.0 mm. Hg with a standard deviation of 2.2. Lilienthal and co-workers (12) using the same method for effective alveolar air and the Riley technique for direct measurement of the arterial gas tensions found an average gradient of 8.9 mm. Hg $\pm$ 2.9 in six normal subjects. Cournand and colleagues (13) with identical methods in 10 normal individuals found an average gradient of 10.0. Comroe and Dripps (14), employing a method for determining blood gas tensions comparable to ours and end-expiratory Haldane-Priestley alveolar air samples, found an average gradient of 0.7 ± 5.3. Barker, Lambertsen and associates (15), using effective alveolar air and an improved Riley method for arterial P_{O_2} found an average difference between the two of 3.7 mm. Hg which was statistically not significant.

TABLE I

Normal alveolar-arterial P_{O_2} gradient **

Case	Barometric pressure	Arterial P_{CO_2} (P_{aCO_2})	A Alveolar P_{O_2} (P_{AO_2})	B Arterial P_{O_2} (P_{aO_2})	A – B Gradient
	mm. Hg	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
1	740	41.2	98.8	93.8	5.0
2	741	37.2	96.0	90.2	5.8
3	750	35.4	102.2	99.8	2.4
4	750	37.1	102.4	99.8	2.6
5	750	38.2	99.2	95.0	4.2
6	742	40.0	99.5	100.0	−0.5
7	747	36.6	108.4	104.4	4.0
8	745	36.9	102.2	98.0	4.2
9	745	38.2	100.5	101.5	−1.0
10	747	41.4	101.5	98.0	3.5
					Av. 3.0 ± 2.2

* Normal males 25–35 years old.

Arterial oxygen saturation

Immediately following the collection of the blood sample for the direct determination of gas tensions a second syringe, the dead space of which was filled with heparin, was attached to the needle and 20 cc. withdrawn for the determination of oxygen saturation in duplicate. The manometric method of Van Slyke was used for oxygen content and the Sendroy technique for capacity (except in the first three patients in whom the usual tonometer method for capacity was employed). Duplicate determinations checked within 0.15 vol.%.

Vital capacity and subdivisions

The measurements of vital capacity, inspiratory capacity and expiratory reserve volume (6) were obtained with the patient supine. Three determinations of each volume were made and the largest value recorded. All volumes shown in Table III are corrected to 37° C., prevailing barometric pressure and saturated with water vapor (BTPS).

Saturation time from room air to 100% oxygen

The time in seconds required for the arterial oxygen saturation to reach a maximum value when the inspired gas was changed from room air to 100% oxygen was de-

TABLE II

Alveolar-arterial P_{O_2} gradient before and after lipiodol

Case Age Sex	Diagnosis	Time	O_2 Saturation	CO_2 Content	Arterial P_{CO_2} (P_{aCO_2})	A Alveolar P_{O_2} (P_{AO_2})	B Arterial P_{O_2} (P_{aO_2})	A – B P_{O_2} Gradient
			%	*mMol. L.*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*
Ha	No pulmonary disease	Before	99.27*	19.29	43.0	88.8	90.3	−1.5
45		5 hours	94.67*	17.81	41.6	99.7	95.2	4.5
M		1 day	95.52*	20.74	43.7	93.9	94.2	−0.2
Co	Bronchitis and slight	Before	97.68*	22.16	40.5	95.6	93.8	1.8
52	emphysema	6 hours	94.05	21.74	38.5	96.6	92.9	3.7
M		1 day	96.62	21.43	38.8	90.6	90.4	0.2
Bo	Bronchiectasis	Before	99.78	20.74	36.8	103.3	102.6	0.7
24	LLL and lingula	4 hours	98.00	21.29	36.1	100.4	97.2	3.2
M								
Beh	No pulmonary disease	Before	97.06	18.95	39.9	92.4	96.0	−3.6
17		5 hours	94.12	19.56	46.4	81.2	80.5	0.7
F		1 day	92.68	21.88	47.7	91.7	89.0	2.7
Bu	Carcinoma obstructing	Before	85.06*	27.22	46.9	92.2	(48)†	44.2
48	R. bronchus	2 hours	86.38*	25.58	41.4	95.2	53.2	42.1
M		1 day	84.95*	27.75	45.6	94.9	56.1	38.7
Bet	Severe emphysema	Before	90.99*	22.48	36.9	98.7	62.3	36.4
41		3 hours	85.32*	25.73	38.5	98.8	57.4	41.4
M		1 day	91.13*	23.62	36.5	95.8	60.3	35.5
Ho	Bronchiectasis L. lung,	Before	93.57	24.20	40.6	91.9	81.2	10.7
56	fibrosis and severe	Before	91.03	24.40	42.0	92.3	81.6	10.7
M	emphysema	7 hours	86.87	24.84	42.1	95.1	52.8	42.3
		1 day	88.65	24.15	36.4	99.9	60.8	39.1
		2 days	92.95	23.30	42.5	89.8	89.9	−0.1
McC	Bronchiectasis	Before	94.20	21.71	41.5	94.9	83.1	11.8
20	LLL and lingula;	5 hours	94.59	21.90	37.3	100.6	82.7	17.9
M	minimal RLL	1 day			42.8	93.1	83.9	9.2
Da	Infected cysts LLL	Before	93.41	24.26	43.3	92.3	85.8	6.5
15		Before	—	—	44.3	98.5	85.7	12.8
F		5½ hours	92.40	22.26	44.3	89.2	82.2	7.0
		1 day	95.28	22.40	42.9	90.4	89.8	0.6
Mo	Bronchiectasis	Control	87.56	25.95	43.9	77.8	49.0	28.8
69	R. lung	Control	92.39	23.52	42.1	104.0	58.9	45.1
M		4 hours	81.60	25.00	42.9	91.1	52.5	38.6
		1 day	—	—	46.7	89.7	56.0	33.7

* Tonometer equilibration for capacity.
† Calculated from saturation as direct determination not satisfactory.

termined with a modified oximeter designed in this laboratory by Elam, Sleator and co-workers (16–18). The earpiece of this apparatus employs a tungsten filament light source and two selenium photocells from which the electrical responses are channeled separately. The first cell which receives transmitted light filtered through a Wratten 29 filter measures changes in the red spectral region and is sensitive to changes both in total hemoglobin content and in oxygen saturation. The infra-red light which reaches the other cell has passed through three thicknesses of Wratten 87 and two thicknesses of Wratten 88. The output of this cell reflects chiefly changes in the total amount of blood in the path and is independent of oxygen saturation above 60%. From the amplified and continuously recorded outputs of the two cells the oxygen saturation of the blood in the optical path can be determined by means of a calibration curve. This instrument is similar in principle to that described by Wood and Geraci (19). The lag of the recorder for full scale deflection is 0.45 seconds. The ear lobe to which the instrument was attached was "arterialized" by histamine iontophoresis using 5 milliamperes of current for one minute on both the anterior and posterior surfaces.

With the amplifications of the two channels set at an arbitrary level that would give an adequate differential between room air and 100% oxygen values the supine subject was allowed to breathe room air through a three-way stopcock and a system of one-way valves using a

TABLE III

Effect of lipiodol on lung volumes

Case	Time	Inspiratory capacity	Expiratory reserve volume	Vital capacity	Vital capacity change
		cc.	*cc.*	*cc.*	%
Ha	Before	3,385	1,059	4,345	
	6 hours	3,385	709	3,965	− 8.8
	1 day	3,385	952	3,810	−12.3
Co	Before	3,010	842	3,731	
	3½ hours	2,823	804	3,680	− 1.4
	1 day	2,910	784	3,758	+ 0.7
Bo	Before	3,560	922	4,300	
	5 hours	3,600	1,002	4,300	0.0
Beh	Before	2,820	697	3,100	
	5 hours	2,410	646	2,740	−11.6
	1 day	2,760	772	3,000	− 3.2
Bet	Before	1,460	635	1,810	
	3 hours	951	476	1,428	−21.5
	1 day	1,128	626	1,721	− 4.9
Ho	Before	1,240	816	1,830	
	6 hours	861	622	1,680	− 8.2
	1 day	880	828	1,811	− 1.0
	2 days	894	894	1,810	
McC	Control	2,605*	1,010	3,620	
	5 hours	2,710	1,003	3,770	+ 4.2
	1 day	3,020	1,069	3,838	+ 6.3
Da	Before	1,058	370	1,320	
	Before	1,430	529	2,118	
	5 hours	1,310	502	1,780	+ 3.6
	1 day	1,486	530	1,911	+11.2
Mo	Before	1,360	532	1,880	
	Before	1,400	635	1,800	
	4 hours	1,100	481	1,380	−25.0
	1 day	1,149	521	1,680	− 8.7

All volumes corrected to 37° C., prevailing barometric pressure and saturated with water vapor (BTPS).

* Control taken seven days after lipiodol.

mouthpiece and nose clip. When the oximeter baseline was found to be stable, turning of the stopcock at the end of a normal expiration allowed the subject to breathe from a reservoir of 100% oxygen and expire into the room. The instrumental dead space was 70 cc. Each period of oxygen breathing lasted from two to seven minutes and between determinations intervals of seven to ten minutes were allowed for the saturation to reach the room air baseline again. In each subject from three to nine measurements of saturation time were made and the results averaged.

The method of interpreting the records requires some comment. Fowler and Comroe (20) in measuring the saturation time used a single-channel Millikan oximeter which partially compensated for changes in total hemoglobin. The instrument was set at 96% saturation with the normal subject breathing room air. Because the moment of reaching maximum saturation when the subject breathed 100% oxygen was difficult to appraise accurately, Fowler and Comroe used as endpoint 0.5% saturation below the highest value. The instrumental lag was 0.07 seconds to reach 95% of the final value. This method of measurement requires the assumptions that identical deflections of the instrument in different individuals represent identical changes in saturation and also that all subjects were 96% saturated while breathing room air.

In our studies we measured the total deflection from room air to 100% oxygen and subtracted 10% of this deflection to obtain an arbitrary endpoint. The time in seconds between the first inspiration of 100% oxygen and this point is called T_{90} or the saturation time.[5] The oximeter was thus used as a qualitative instrument only.

As a corollary of the above measurement, the lung-to-ear circulation time was also obtained from the records by measuring the interval from the first inspiration of 100% oxygen to the first upswing of the saturation curve recorded at the ear.

RESULTS

Alveolar-arterial Po_2 *gradient*

From our control studies on normal individuals an alveolar-arterial Po_2 gradient of less than 7.4 mm. Hg can be expected in 95% of normal individuals (mean plus 2 x standard deviation). Considering this value as the upper limit of normal it was found that out of 10 patients studied all but four had abnormally large gradients, ranging from 9.7 to 44.2 mm. Hg at rest before lipiodol. This was associated in all instances with a normal "effective" alveolar Po_2. This increase in gradient can be considered as the result of an abnormally large contribution of mixed venous blood to the systemic arterial blood. This large "effective venous admixture" might result either from the presence of a significant number of perfused but nonventilated alveoli or from impaired diffusion across a pathologically thickened alveolar membrane with or without adequate ventilation. Arterial oxygen saturation less than normal (93%) was found in the four cases with the greatest gradients. These data are summarized in Table II.

After the administration of lipiodol the Po_2 gradient increased by 6 mm. Hg or less in all but two instances. These changes and that occurring

[5] Our data are nevertheless roughly comparable to those of Fowler and Comroe (20) for the following reasons. These authors noted a mean increase of 3.72% saturation. From this 0.5% was subtracted for the endpoint which thus occurred 3.22% above the room air level. This is 3.22/3.72 or 86% of the total deflection which compares quite closely with our endpoint (90% of total deflection).

in Da (a *decrease* of 2.7 mm. Hg) are of questionable significance since they are close to the limit of experimental error. In only one patient (Ho) was a significant increase in Po_2 gradient found (31.6) and this persisted until 48 hours after the oil was given. This 56 year old man had advanced bronchiectasis of the left lower lobe and lingula associated with considerable emphysema and fibrosis. The change in his arterial oxygen saturation (5.4%), although not the largest in the series, reduced it to a definitely anoxic level from a control value close to normal. This individual was the only one of the six in this series who eventually underwent pulmonary resection who did not survive the postoperative period. He died on his fourth postoperative day of respiratory insufficiency. The remaining patients survived operation without unusual respiratory symptoms. Mo, who appeared to be just as poor an operative risk, had only a minimal change in alveolar-arterial gradient after lipiodol although the reduction in his vital capacity was the largest in the series (460 cc.). Statistical analysis of the data as a whole, using Student's t-test indicates no significant change in alveolar-arterial Po_2 gradient nor in absolute value of arterial Po_2 after lipiodol administration.

From the above findings the conclusion can be drawn that the presence of deficient alveolar function *per se* does not necessarily predispose to further impairment by lipiodol, but when this does occur it may be an ominous sign.

As might be expected from the greater diffusibility of carbon dioxide, the data reveal no significant increase in arterial Pco_2 following lipiodol except in one case (Beh). As this patient had no demonstrable lung pathology the finding is probably due to experimental error.

Arterial oxygen saturation

Although some cases had a fall in saturation as large as 5% after lipiodol most values remained within the range of normal (*i.e.*, above 93%). Three patients (Bet, Mo and Ho) did develop a significant degree of desaturation after bronchography, but in the former two this was not associated with a significant change of arterial Po_2 or of alveolar-arterial Po_2 gradient. Although the change in oxygen saturation occurring within several hours after bronchography was found to be statistically significant, it should be pointed out that duplicate determinations may differ by as much as 2% saturation.[6]

Vital capacity

Statistical analysis of the vital capacity data (Table III) indicates a significant reduction ($P < .05$) from the control value on the day the bronchograms were made (average reduction 7.6%) but not on the following day (average difference 1.5%). Individually two cases showed a slight increase in vital capacity after lipiodol, two showed minimal or no change and four had from 8–25% reduction within several hours with a return to within 9% or less of the control value by the following day. Only one case (Ha) showed a progressive decrease in vital capacity amounting to a maximum loss of 12.3%. This man, however, had no demonstrable pulmonary pathology and his cooperation was open to question.

Oximeter data

The results of the studies of saturation time are shown in Table IV. In the four normal cases

[6] For this reason and because of the nearly horizontal course of the oxygen dissociation curve in the high range of oxygen saturation, derivation of oxygen tension from oxygen saturation in this range is not permissible and the direct measurement of tension becomes a more sensitive index of changes.

TABLE IV

Saturation time in normal subjects and patients before and after lipiodol

Normal subject	Saturation time	Patient	Saturation time			B – A
			A Before	B After 2–7 hrs.	C After 24 hrs.	
	seconds		*seconds*	*seconds*	*seconds*	*seconds*
GB	31.5	Bu	86.6	53.9	47.1*	−32.7
GB	26.4	Ha	22.1	19.7	18.0	− 2.4
AR	22.5	Bet	31.0	32.3	51.5	1.3
AR	22.4	Co	44.5	44.6	38.9	0.1
AR	29.4	Ho	82.4	101.9	132.2	19.5
					81.9†	
AR	28.4	Bo	23.8	19.2	—	− 4.6
RP	24.6	Da	24.6	—	—	—
WE	24.0					
Mean	26.2		45.0	45.3	57.5	− 3.2
S.D.	3.4		28.0	30.3	43.7	

Each value in this table represents the average of from two to five readings taken consecutively on the same day.
* Two days after lipiodol.
† Four days after lipiodol.

considerable variability was found, the greatest difference between readings at a single session in the same subject being 17.0 seconds. The mean normal saturation time was 26.2 (±3.4 seconds). This compares closely with the average of 15–30 seconds found by Elam and colleagues (17) with the same equipment and a mean of 25.0 found by Wood, Taylor and Knutsen (21) with their instrument. Using the Millikan oximeter Fowler and Comroe (20) found a mean of 52.0 seconds ± 18.6 and Douglas and Edholm (22) 58.4 ± 6.1.

In the six patients studied a mean saturation time before lipiodol of 45.0 seconds ± 28.0 was found. The reproducibility of the data was comparable to that in the normal subjects except in one case in which a difference of as much as 47.0 seconds was found between successive readings. The administration of lipiodol did not result in a consistent change in saturation time, the mean change amounting to − 3.2 seconds.

It should be emphasized that the saturation time is influenced by several mechanical and physiologic factors other than the transport of O_2 across the alveolar membrane. Among these are proper timing of the beginning of inspiration of O_2, efficiency of intrapulmonary mixing, circulation time from lung to ear and instrumental factors including changing ear lobe volume as the edema from histamine subsides. Inability to control these rigidly undoubtedly accounts for the poor reproducibility.

Measurements of the lung-to-ear circulation time was also obtained from the records. In normals a mean of 5.4 seconds ± 0.7 was found. The mean for the patients (6.6 seconds ± 2.0) was not significantly different. No prolongation of circulation time after lipiodol was observed. (Average several hours after bronchography 6.5 ± 1.1; one day afterwards 6.2 ± 1.7.)

DISCUSSION

From our studies of the alveolar-arterial P_{O_2} gradient the conclusion can be reached that in most instances lipiodol in the amounts commonly used does not increase the proportion of mixed venous blood reaching the arterial circulation ("effective venous admixture"). It, therefore, neither decreases alveolar ventilation nor does its presence on the alveolar surface form a significant barrier to diffusion of oxygen. Even in those cases with an abnormally high control gradient, lipiodol does not generally appear to add further to the abnormality. This applies both to patients with diffuse pulmonary disease (emphysema) as well as to those with more localized processes such as parenchymal infiltration or atelectasis. The pulmonary pathology of the one case in our series with a considerable increase in gradient following lipiodol was not fundamentally different from that found in the other patients, except perhaps in extent. For the purposes of evaluating the advisability of delaying operation after lipiodol administration, however, it should be noted that even in this individual the gradient returned to the control level within 48 hours. In addition, all abnormal values found on any of the tests in the remaining patients had returned to the pre-lipiodol level within 24 hours.

It must be realized that varying amounts of bronchial secretion may result in spontaneous fluctuations in the oxygen gradient. Such fluctuations have actually been demonstrated in some of the patients in whom more than one control study was done. Any comparison between studies before and after lipiodol should be considered in the light of this varying baseline.

In evaluating our data on vital capacity and subdivisions comparison with the work of Zavod (23) can be made. This author found in 50 patients an average loss of 15% of vital capacity within one hour of the administration of lipiodol. This loss decreased to 10% on the following day and had disappeared by the fourth day. It was more at the expense of expiratory reserve volume than of inspiratory capacity. A similar trend is found in our data although the losses of vital capacity are not as marked and not as consistent. We are reluctant to attach any importance to the immediate reduction in vital capacity since it may be due to inadequate cooperation of the patient in whom the presence of residual lipiodol often precipitates cough on maximal expiratory effort.

In summary, we may conclude that the fear of further impairment of alveolar function need not delay an intrapleural operation more than a few days after bronchography.

CONCLUSIONS

1. A study has been made of the pulmonary function of 10 patients before and after bronchography by means of lipiodol.

2. In only one patient was the alveolar-arterial Po_2 gradient found to be definitely increased following the procedure, although five cases in addition to this one had an abnormally high gradient in the control study.

3. Arterial carbon dioxide tension was essentially uninfluenced by lipiodol.

4. The vital capacity was reduced by an average of 7.6% several hours after bronchography.

5. Measurement of the saturation time on changing the inspired gas from room air to 100% oxygen revealed no significant effect after lipiodol. Lung-to-ear circulation time was similarly unchanged.

6. All abnormal values returned to the control level within 24 hours except in one case in whom the pre-lipiodol values were not reached until 48 hours later.

7. It is concluded that the presence of lipiodol has no significant influence on the respiratory gas exchange except in occasional patients with advanced pulmonary pathology.

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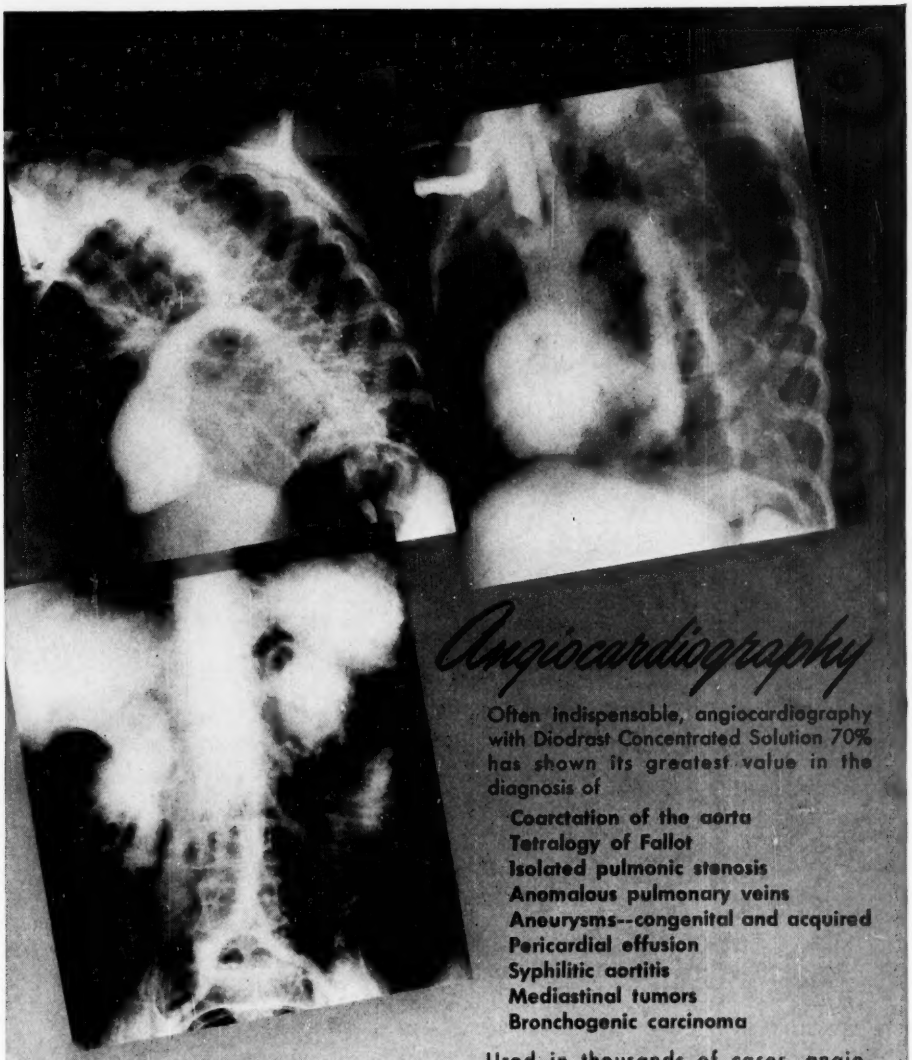

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